

Sad Case of Dr Zhores Medvedev

DR ZHORES MEDVEDEV's truce with the Soviet authorities has come to an inglorious end. What most informed western observers had foreseen and, more than likely, what Dr Medvedev had feared, came to pass. By what can be called nothing but trickery Dr Medvedev and his wife were twice summoned to the Soviet Embassy in London last week and during the second visit the true reason for the summons was revealed and Dr Medvedev was informed that his Soviet citizenship had been cancelled and his passport, which he was told belonged to the state, had to be surrendered.

In retrospect no one should be surprised by this action, for apart from adding another chapter to the already chequered career of Dr Zhores Medvedev the move is entirely in keeping with the character of the Soviet authorities. In recent months *Nature* has more than once taken note that Soviet scientists are being victimized, for all scientists, whether they are practising their professions in the western world or beyond the Iron Curtain, should be concerned when colleagues are being persecuted. In this respect it has to be said, in answer to those who have taken *Nature* to task recently for highlighting the fate of Soviet scientists while apparently ignoring the fate of other persecuted intellectuals, that the journal is not a platform for discussing all aspects of human rights. Nevertheless *Nature* must take note when scientists, from any country, are persecuted because of their profession. Since coming to Britain in January, Dr Medvedev has neither discussed publicly his previous treatment by the Soviet authorities—as well he might have—nor has he in any way criticized his government's policies. His one public statement, made in the wake of last week's events, is factual and contains no adverse comment and it is clear that he is still determined to return to the Soviet Union to continue his scientific work. It is indeed the duty of his fellow scientists to help him and the question is how best to do this.

It is paradoxical that while the Soviet authorities are faced with several dissident Jewish scientists trying to emigrate to Israel, Dr Zhores Medvedev is deprived of the basic human right of being able to return to live in his own country. But the Soviet Union was one of the countries which did not vote in favour of the United Nations Declaration of Human Rights and so, presumably, it does not feel bound by the article in the declaration which states that everyone has the right to leave and to return to his own country. In the long run it would be a laudable aim to get the Soviet Union to approve this declaration, but in the short term this is not going directly to benefit Dr Medvedev or those potential emigré scientists. There is a campaign in progress to help Jewish scientists leave the Soviet Union although its successes, so far, are limited. This campaign is based on maximum publicity in the western press coupled with well publicized hunger strikes and demonstrations interspersed with frequent, communal, telephone calls between west and east.

But how should Dr Zhores Medvedev set about regaining his lost citizenship? At first sight it would appear that a publicity campaign directed by and centred around Dr Medvedev himself would be counter-productive and would firm the resolve of the Soviet authorities to banish him. Conversely no campaign at all with Dr Medvedev remaining quietly in the background, as he has done for the past seven months, hoping against hope for a change of heart and awaiting a call from the embassy, would seem to be even more hopeless.

This, in essence, is the dilemma in which Dr Medvedev finds himself at present. And it seems that help for him must come from the scientific community which must be prepared to act without any overt encouragement from Dr Medvedev. But what is there to be done? Throwing stones through embassy windows is an exercise which after the first flush of anger and enthusiasm has worn off would seem to be futile. Is this the time perhaps when invitations to Soviet scientists to attend conferences in the west should be withdrawn and participation in exchange schemes temporarily halted? Drastic measures indeed, which might damage several years of careful nurturing of Western-Soviet relations, but this is a drastic situation. In the end it is only the scientific community that can decide the extremes to which it wants to go to help a colleague in distress. And in this case it must not get too discouraged if there is an apparent lack of interest and cooperation from Dr Medvedev himself.

100 Years Ago



Although, as the writer observes, "in cold weather the impulse to act is much more powerfully felt than in summer, when the air is hot, and therefore the temperature of the surface is higher," yet even 98° F. (the internal temperature of a healthy body) is not uncommon for the air in tropical climates, where the natives can undergo great exertions. But, according to the thermo-electric hypothesis, the nerve force must in this case be *nil*. Again, temperatures of 140° to 160° F. are easily sustained for a considerable time in the Turkish Bath. Under these conditions the direction of the current should be reversed; and, even supposing that positive and negative currents both acted in the same sense on the muscles and nervous ganglia, it would seem that there must be an instant of transition when the two should be balanced, and nervous force at zero, and the powers both of sensation and motion lost with it.

From *Nature* 8, 324, August 21, 1873



OLD WORLD

Medvedev still Determined to be a Soviet Citizen

DR ZHORES MEDVEDEV, in spite of the fact that he has been deprived of his Soviet citizenship, is still determined to return to live in the Soviet Union. Earlier this week he said that he would appeal against the decision of the Praesidium of the Supreme Soviet made on July 16 which was based on Dr Medvedev's apparent violation of a decree passed in 1937 which allows Soviet citizens to be deprived of their citizenship if they break certain rules which apply to Soviets living abroad.

In a statement issued at the end of last week, Dr Medvedev points out that it is usual for Soviet citizens who travel abroad to sign a declaration undertaking not to violate these rules and regulations. But Dr Medvedev was neither shown the regulations before leaving the Soviet Union nor was he asked to sign any declaration. He therefore argues that he cannot be responsible for regulations he has neither seen nor signed.

Dr Medvedev's continued hope that his citizenship will be restored and his passport returned is based on a discussion he had last week with the secretary to the consular department at the Soviet embassy. This gentleman told Dr Medvedev that the decision was not irreversible and that an appeal could be made through official channels. But in the same conversation Dr Medvedev was informed how to obtain documents from the British Foreign Office to enable him to travel outside Britain.

Dr Zhores Medvedev's difficulties with the Soviet Authorities first became evident after he attended a meeting in Paris in 1957. Soon after this, his first meeting outside the Soviet Union, he was inundated with requests to participate in meetings and research projects, and to deliver lectures abroad, but all his applications for foreign travel were turned down until late last year. During these long years Dr Medvedev continued with his research work and wrote several books, the best known of which is *The Medvedev Papers*, published in 1971.

Since then Dr Medvedev has had an even more difficult time with the Soviet Authorities. In July 1972 he was ignominiously sent home from Obinsk by the Soviet police and refused permission to attend an International Congress of Gerontology which was being held in that city. And during 1972, after receiving an official invitation from the National Institute for Medical Research to spend a year in London working at the institute's Mill Hill laboratories, his application for permission to take up this appointment was at first turned down.

But in December of last year, Dr Medvedev was granted a passport and he arrived in London early this year.

During the past seven months Dr Medvedev has deliberately kept out of the limelight and he has rejected advances from the press for interviews and he also refused to take part in any political activity. The one action which might have been the reason for the Soviet Praesidium depriving him of his citizenship was his signing of an agreement to publish in Britain his book, *Ten Years After Ivan Denisovich*, which was written before he came to Britain. But Dr Medvedev is adamant that the book can never be used against him in the Soviet courts for it "is not political and criticizes neither the Soviet system nor the Soviet government". The book, says Dr Medvedev, "gives only a factual account of events that actually took place and cannot be considered in any way as

slandrous, defamatory or anti-Soviet". This book, in fact, gives an account of how Alexander Solzhenitsyn has been treated by the authorities since he published his first novel, *A Day in the Life of Ivan Denisovich*.

Dr Medvedev's twin brother Roy has issued a statement in Moscow deploring the treatment accorded his brother and he describes the deprivation of citizenship as an "absurd act". According to other reports from Moscow Dr Medvedev has lost his citizenship as a result of "actions discrediting the high title of citizen of the USSR". According to Tass, the Soviet news agency, the action by the Praesidium was taken under an article of the Soviet Legal code which deals with anti-social acts or defamation of the Soviet state. Tass also added that Dr Medvedev had fabricated slanderous materials discrediting the Soviet state and social system.

NORTH SEA RESEARCH

Caught up in the Flow

ON September 10 some ninety current meters, twenty bottom mounted offshore tide gauges and three automatic buoys will be busily recording currents and tides in the southern North Sea, while more than fifty research institutes and laboratories in Belgium, the Netherlands and Britain will be waiting for the results.

The operation, entitled JONSDAP 73, is intended to provide a whole host of basic data about the North Sea which can be used to produce numerical models for predicting the behaviour of one of the busiest stretches of sea in the world.

JONSDAP 73 (Joint North Sea Data Acquisition Programme) is the brainchild of a body calling itself JONSIS (Joint North Sea Information Systems) which got under way in 1970 when the West German oceanographers approached the Natural Environment Research Council to suggest an exchange of information about their research in the North Sea. Since then, Belgium, the Netherlands and Sweden have joined the club, and JONSDAP is the first specific project to emerge. It consists chiefly of rephased programmes of the various bodies involved which have been brought together to provide one mass of data in an exercise which is planned to last at least 40 days and which will involve twenty-two research vessels.

British institutions involved include the Institute of Oceanographic Sciences,

the Fishery Laboratories at Lowestoft, the Universities of Bath, East Anglia and Liverpool, the Port of London Authority and the Hydraulics Research Station. Research projects on tides, storm surges, current vectors, circulation and mixing and pollution problems should all benefit from the studies.

Whereas the chief object is to provide a wealth of basic data, each country and institute has its own particular interest. The Belgians and the Dutch are particularly interested in the pollution aspect of the studies—measurements of turbidity, salinity, temperature, trace metals, the release of drifters and diffusion of rhodamine dye experiments are all part of the programme—while the Institute of Oceanographic Sciences is aiming to find out more information about storm surges, particularly with a view to the development of a Thames barrier to save London from flooding.

One of the more interesting parts of the study will be an attempt to monitor flow through the Straits of Dover using a cross-Channel telephone cable. The technique involves sensing and interpreting the electrical signal induced by the movement of seawater through the Earth's magnetic field, and once the cable is calibrated it should be possible to continue to use it as a flow meter.

If the operation is a success a large bank of data should be available by the end of 1974. And further work is planned. West Germany and Sweden are not involved in this particular operation, but a similar exercise is planned for the northern North Sea in 1975.

ENGINEERING

Total Technology

A LIMITED but enthusiastic backing for Total Technology emerges from a Science Research Council report published last week. Total Technology—one of those multi-disciplinary concepts that industry advocates and universities abhor—is the brainchild of Professor Hugh Ford of Imperial College London, who chaired the panel that has now recommended that PhDs in total technology should be awarded in the near future, with the intention of producing about 20 doctorates a year in the subject.

This course of action is rather more modest than the approach suggested by an earlier SRC working party on the subject chaired by Professor J. H. Horlock. His group's work laid the ground for Professor Ford's panel, but Horlock's recommendation was that at least 25% of postgraduate engineering students supported by the SRC should receive training in Total Technology by the end of the decade. In real numbers this would be about 200 PhDs a year.

But what is Total Technology? The report explains it by stating that the practice of engineering comprises research, development, design, production, marketing and operation of plant, while the service and construction industries require a special emphasis on planning and operations management. Total Technology is all these functions merged together to produce success.

What that means in practice is likely to be discovered this autumn at the Universities of Cambridge, Strathclyde and Aston where the first courses are to be run. The basic emphasis is to build up an appreciation of managerial functions combined with the ability to communicate with others during work as an engineer.

Inevitably this implies a deal of course work. But Professor Ford and his panel are adamant that they are not proposing the award of a PhD for purely course work. In the outline syllabus that is appended to their report the panel—which was drawn from industry as well as the universities—proposes that the three year doctorate should cover course work on the chief sectors of industry from raw materials to primary and secondary industry, large-scale manufacturing, mass production and small specialized industries, combined with detailed work on two background disciplines chosen from such areas as design function, industrial sociology, the economics of companies, systems analysis and plant maintenance.

This part of the course might be compressed into one year to become a master's degree, the panel suggest. But the really meaty part of the operation—the part that the panel maintain makes

the operation worthy of a PhD rather than just a master's degree—is work on industrial projects. These should extend over the last two years of the course, comprising one, two or possibly three open-ended projects broadly associated with the subjects studied earlier. The choice of subject would be made in collaboration with industry. "The student would thus" the panel say, "attack an industrially relevant problem, of direct interest to the firm, and obtain a solution in a realistic time".

Clearly the panel is convinced of the value of what it recommends. But what does industry think? The reaction to Professor Horlock's original proposals was a little cool. The relevance of existing PhDs was eagerly attacked, and Total Technology as an idea was warmly received, but industry doubted whether the target of 25% of SRC-supported engineering PhDs being total technologists by the end of the decade was a desirable or even a possible target, and the old bugbear of producing more doctorates than could be employed was raised. A one year MSc was thought to be a more preferable alternative.

The result of all this is that Total Technology will appear, but slowly. The panel stresses the difficulties of intro-

ducing the subject and emphasizes that the new courses must benefit from the experience gained by their first students. A gradual introduction is more likely to produce a course that produces the type of doctorate that industry wants.

NAUTICAL ARCHAEOLOGY

Protecting Wrecks

from a Correspondent

THE first sites of wrecks to be given protection under the recently passed Protection of Wrecks Act were announced last week by Mr Cranley Onslow, Under Secretary of State for Aerospace and Shipping. The act provides for specific wreck sites of cultural interest within British territorial waters to be given something of the status of an ancient monument on land.

British wreck sites are typically collections of related objects rather than well-preserved ancient hulls, which, paradoxically, are found in river banks, marshes and harbours. And three of the four sites initially designated by Viscount Runciman's committee will be just such a collection of historical objects.

The four sites are the royal yacht, Mary, a gift from the Dutch to Charles II and wrecked off Anglesey in 1675; the Dutch East Indiaman, Amsterdam, beached near Hastings when outward bound on her maiden voyage in 1749; Henry VIII's Mary Rose, capsized near Spithead in 1545 and preserved to her gundeck in seabed silt, and the Grace Dieu.

The Mary Rose is thought by the survey team currently working on her to be sufficiently well preserved to be raised whole. The construction of the boat is of great interest but the prospect that her contents might be recovered—the Solent silt is a good preservative—creates even more excitement. The Mary Rose is known to have sunk during a battle manoeuvre, thought to have been planned by King Henry VIII himself who watched the scene from the battlements of Southsea Castle, so the possibility is high of there being documents of great historical interest on board.

The fourth candidate, however, is a very special case. Henry V's Grace Dieu—a ship from the early 1400s—was built for wars that were over by the time she was ready. The largest ship built in Europe for over 200 years, she has lain in the tidal Hamble river since she sank. It is thought that only five feet of her timbers above the keel are left, for, in spite of being largely submerged, she has been the object of souvenir hunters for centuries and is now threatened by a motorway. Nevertheless those five feet are of compelling interest, for they are of unique triple-clinker construction.

Nature Subscriptions

FROM the first issue in 1974, *Nature* will appear in a single weekly edition of about the same number of pages as the present three weekly editions taken together. The single weekly edition will replace the present Friday *Nature*, *Nature New Biology* and *Nature Physical Science*.

The decision follows comprehensive discussions with contributors and readers all over the world. It reflects the general belief that a new, unified *Nature* will provide a better service for the scientific community.

Annual subscriptions, despatched by surface mail, will be £22 in the United Kingdom and elsewhere, except in the United States and Canada (£28). Cover price in the United Kingdom will be £0.35.

Nature has had to absorb steeply rising costs on production, paper and despatch. But, for the majority of subscribers, the new price will mean they actually pay less for *Nature* in 1974 than they are paying in 1973.

Subscribers will receive full details with their renewal notices later this year.—D. D.

Dr David Davies takes up the editorship of Nature next Monday, August 20.

NEW WORLD

Vitamins, Safety and Freedom of Choice

by our Washington Correspondent

IN the past two years, the so-called health food industry has been going through a period of burgeoning growth in the United States. Founded on sound nutritional science and sheer quackery in varying proportions, and aided by growing public distrust of modern agricultural and food processing practices, the industry has managed to remain relatively free from government control in general and from the tiresome task of having to justify some of its more excessive advertising claims in particular. But, if the Food and Drug Administration gets its way, the health food industry is heading for a somewhat dramatic change.

Earlier this month, the FDA published a set of regulations governing the sale of vitamins and minerals and the fortification of foods which, barring successful challenge in the courts or in Congress, will force some radical changes in the promotion, packaging and sale of health foods. The regulations have, however, stirred up something of a hornets nest for they have been bitterly attacked by a variety of people and they have caused confusion and outrage. They clearly throw up some important philosophical questions.

The new regulations took ten years to draw up and are due to be enforced at the beginning of next year. In short, they will chiefly affect the sales and promotion of large doses of vitamins and minerals, but they will not greatly affect the marketing of such products in doses that the FDA reckons the average healthy person needs each day to maintain good nutrition. The key to the regulations is a switch from the Minimum Daily Requirement as the official measure of nutritional intake to a new standard called the US Recommended Daily Allowance (RDA). The old standard was simply the minimum level of each nutrient required to prevent the onset of deficiency diseases, while the RDA, which is based on criteria suggested by the National Academy of Sciences, is supposed to represent levels required for good nutrition.

The FDA has established RDAs for nineteen essential nutrients, and it is attempting to draw distinctions between ordinary foods, special dietary supplements and drugs, on the basis of the percentages of the amount of RDA they contain. In general, if a product contains less than 50% of the RDA per serving, it will be classed as a food, if it contains between 50% and 150% of the RDA, it will be classed as a dietary

supplement, and those products containing more than 150% of the RDA will be classed as drugs.

Different regulations will apply to each category, but the chief effect will be that manufacturers will have to amend advertising claims, label their products appropriately, reformulate some dietary supplements to get rid of irrational combinations of vitamins and minerals and end the practice of claiming nutritional value for essentially worthless ingredients. Foods which contain less than 50% of RDAs, for example, cannot be marketed on the basis of claims of therapeutic value, while heavily fortified breakfast cereals will have to be marketed as dietary supplements rather than as foods. But as for vitamins and minerals sold in high dosages, they will eventually have to meet standards of efficacy and safety that apply to all drugs.

The idea is not, however, to force high dosage products off the markets, or even to stipulate that they should only be made available on prescription. This, in short, is what will happen. Vitamins and minerals which contain more than 150% of the RDA will, from January 1,

1974, have to be sold as drugs, bearing labels stating that they should be taken for therapeutic purposes only. With the possible exception of vitamins A and D, they will still be available in drug stores without prescription, but eventually they will all come under the general review of over-the-counter drugs which is being conducted by the FDA, and they will have to meet stringent tests of efficacy and safety. In other words, nobody will be prevented from taking large doses of Vitamin C to guard against the common cold, provided that such large doses are found to be safe and effective. (In any case, the vitamin will still be available in small dose units which could be taken by the handful.)

As for vitamins A and D, the FDA is proposing to limit high doses to prescription-only status because of their toxicity. High doses of Vitamin A taken over long periods can increase pressure within the skull and mimic a brain tumour, and they have been found to retard growth in children. Excessive doses of Vitamin D have also been shown to retard mental and physical growth in children.

Why should the FDA bother to

Education Budget

by our Washington Correspondent

PRESIDENT Nixon last week announced that he has nominated Dr Lowell J. Paige, Dean of Physical Sciences and Professor of Mathematics at the University of California, Los Angeles, to be the new Assistant Director of the National Science Foundation for Education. The job has been vacant for nearly two years, since Dr Lloyd G. Humphreys abruptly resigned in September, 1971, in protest because the Office of Management and Budget had impounded some \$30 million earmarked for the NSF's education and institutional support programmes, and because he feared that even deeper cuts were on the way.

His prognosis was correct, for the foundation's budget for science education improvement sank from \$73.4 million in 1972 to \$47 million in 1973—again, with considerable impounding of funds by the Office of Management and Budget—and the Administration has continued its plans to phase out graduate training programmes and cut back on graduate fellowships. While the funds were being cut, however, the foundation

drastically revamped its educational support programmes, concentrating on four chief areas: a scientific literacy programme, aimed at increasing the scientific education of all students, not just those intending to follow careers in science; a programme aimed at increasing the effectiveness of science teaching at all levels through support of innovative courses; a programme of research on science education to determine which elements of the programmes are successful; and the development of courses and curricula centred around problems which span several disciplines.

The cutbacks of the past two years have evoked howls of protest from the universities and from the House Committee on Science and Astronautics, which oversees the work of the NSF. But there are, however, signs of improvement, for the foundation's educational support budget is set to increase to \$60 million this year, and the chances are that the upward trend will be maintained. But the most hopeful sign is that the Administration has at last decided that the NSF's education programmes are sufficiently important to require a permanent director.

regulate the health food industry, in any case? One reason is that the sale of vitamin and mineral products in the United States now runs into hundreds of millions of dollars a year, and they find their way into some 75% of US households. Moreover, there is a growing tendency to fortify foods with vitamins and minerals with an accompanying blast of promotion drawing attention to the fact. Some breakfast cereals, for example, have been turned into the equivalent of high-calorie vitamin pills, and they will now have to be recognized as such. The FDA thus argues that since the consumer is usually in no position to judge which formulations are based on sound nutritional science and which are inherently irrational, the door is wide open for outright deception; some protection from exploitation, unsubstantiated advertising claims and possibly harmful doses of nutrients is therefore required.

But, as a flood of letters, postcards and verbal complaints has demonstrated, not everybody agrees. In particular, the new regulations have met with generally favourable reactions from consumer advocates, and some nutrition scientists, but with vehement opposition from others. Moreover, a bill introduced into the House of Representatives by Craig Hosmer, a Republican from California, which attempts to stop the FDA from regulating vitamins and minerals for reasons other than safety, has gathered 160 cosponsors.

One person who is especially upset by the regulations is Dr Linus Pauling, the Stanford biochemist who advocated, in a best-selling book, *Vitamin C and the Common Cold*, the taking of large doses of Vitamin C to guard against colds. Pauling said in a telephone interview last week that he believes the new regulations "will be to the detriment of the health of the American public", and that they are also inherently inconsistent. His chief complaint is that the FDA is attempting to regulate products for which there is little or no concern over safety, and that the regulations "infringe the rights of people to decide what they want to eat".

Pauling pointed out that it is inconsistent for the FDA to regulate vitamin C and not sucrose. Both occur naturally and both are sold in large amounts as purified foods, but the difference is, he said, that vitamin C is essential to life, while sucrose is not. There is, however, considerable evidence of harmful effects derived from large doses of sucrose, while there is virtually no evidence of harm from large doses of vitamin C. "Just because a small amount of ascorbic acid (vitamin C) is needed for life is no reason to classify it as a drug", he said. His argument is that provided there is no proof of harmful effects from taking vitamins, consumers

should be free to choose what they want to eat without government interference.

But not all nutritionists share Pauling's views on the matter. Dr D. M. Hegsted, of Harvard University, for example, said last week that he "cannot, frankly, see much to disagree with" in the regulations. "Somebody", he said, "should have the authority to keep the quackery at a minimum". And his views are shared by some consumer interest groups. In particular Anita Johnson, an attorney with the Health Research Group, a public interest association linked with Mr Ralph Nader, pointed out that consumer advocates "have been supporting the proper testing of foods and drugs and therefore we support the idea to restrict the amounts of vitamins and minerals to levels that we know are safe". And as for the FDA itself, Dr Alexander Schmidt, the new Commissioner of Food and Drugs, pointed out, when the regulations were announced earlier this month, that the Food, Drug and Cosmetic Act of 1938 requires the FDA to insure honest and full consumer information about such products.

Pauling is, however, not simply concerned about infringements of individual liberty, for he believes that the RDAs established by the FDA may be too low to ensure adequate nutrition in a proportion of the public. In particular, he cited a series of experiments performed by Dr Roger J. Williams of the University of Texas which indicated that inbred rats and guinea pigs differ in their requirements for nutrients by factors up to 40. He then suggested that man's requirements may similarly differ from individual to individual, and thus questioned the validity of establishing a standard which allows variation by only a factor of 3.

What are the chances that the opposition to the regulations will force a change in them? One possible area for forcing a change is the Congress, but although Hosmer's bill has garnered 160 cosponsors, it was first introduced in 1967 and has never been taken up by the Commerce Committee to which it was referred. Moreover, there is as yet little evidence of support for the bill in the Senate. Another possibility is a legal appeal. Pauling said last week that he has been talking to lawyers, exploring the possibility of a law suit against the FDA. Similarly, the health foods industry can be expected to challenge the regulations in the courts. But, in view of the FDA's clear mandate to ensure accurate labelling of foods and drugs, the chances that such suits will succeed is generally considered slim.

One ironic footnote to the debate about vitamins and minerals is that for the past few years, the health foods industry has, in large measure, been

spawned by the consumer movement. The effective attacks that consumer advocates have mounted on deceptive packaging, potentially harmful additives, and lack of food value in many popular foods has caused many people to jump on the vitamin bandwagon. Now, however, the wheel has turned full circle and the health foods industry is under attack for many of the abuses that it purports to eschew.

NUCLEAR SAFETY

Long Awaited Inquiry

by our Washington Correspondent

A DATE for the Joint Committee on Atomic Energy's long awaited public inquiry into the safety of nuclear reactors has at last been set. The committee plans to hold three days of hearings on the matter on September 25-27, when witnesses from the Atomic Energy Commission, the Advisory Committee on Reactor Safeguards, the Atomic Safety and Licensing Board and other government agencies will present their side of the case. Those hearings will be followed by another set in the autumn, when public witnesses will have a chance to air their views.

First promised for late last year, the hearings have been put back several times because the Joint Committee has been waiting for the AEC to produce a report on the safety of light water cooled reactors. The report, the size of a telephone directory, was finally delivered to the committee last month after having been reviewed by several groups and individuals, including some of the nuclear industry's strongest friends and harshest critics. It is a candid, comprehensive account of safety questions and the commission's response to them, but the report, known as WASH-1250, also meets some of the most prominent critics head-on in a chapter added to earlier drafts. The chapter includes a short discussion of emergency core cooling systems, and it also tackles some of the questions about low level radiation raised in the past few years by Professor Sternglass and Drs Gofman and Tamplin. Not surprisingly, the report concludes that such critics are incorrect.

The Joint Committee sessions will also explore the ramifications of recent organizational changes in the agency which took nuclear safety research out of the division of reactor development, and which ultimately led to the resignation of Mr Milton Shaw, one of the most powerful AEC officials for the past few years (see *Nature*, 243, 431; 1973). It remains to be seen, however, whether the committee, which is strongly pro-nuclear, can provide the searching inquiry into reactor safety that many groups and individuals are demanding.

NEWS AND VIEWS

Historical Records of British Earthquakes

IF asked, most people, including scientists, would almost certainly pronounce Britain to be fortunately free from the hazard of earthquakes; and if they were judging Britain by the standards of the San Andreas fault, the Japanese trench and other sections of the system of world-wide earthquake belts so elegantly defined by recent observation and plate tectonics they would have expressed a reasonable opinion. But they would not be literally correct, for not only is Britain earthquake-prone, earthquakes occur quite frequently. The late Charles Davison listed no less than 1,191 genuine earthquakes from the year AD 974 when, according to Symeon of Durham "a great earthquake took place over all England", to January 26, 1924, when Hereford was afflicted by an earthquake for the twentieth time in 70 years.

Of course, most of these events were relatively small, but quite a number reached intensity VIII on the Rossi-Forel scale (VII–VIII on the Modified Mercalli scale) and one even reached intensity IX ("chimneys thrown down and cracks made in the walls of about one-half the houses in one place" according to Davison's simplified form of the Rossi-Forel scale). In other words, though Californians, Japanese and Sicilians might be entitled to laugh at the idea of British earthquakes, there can be no doubt that many British cities are susceptible to an unpleasant amount of earthquake damage.

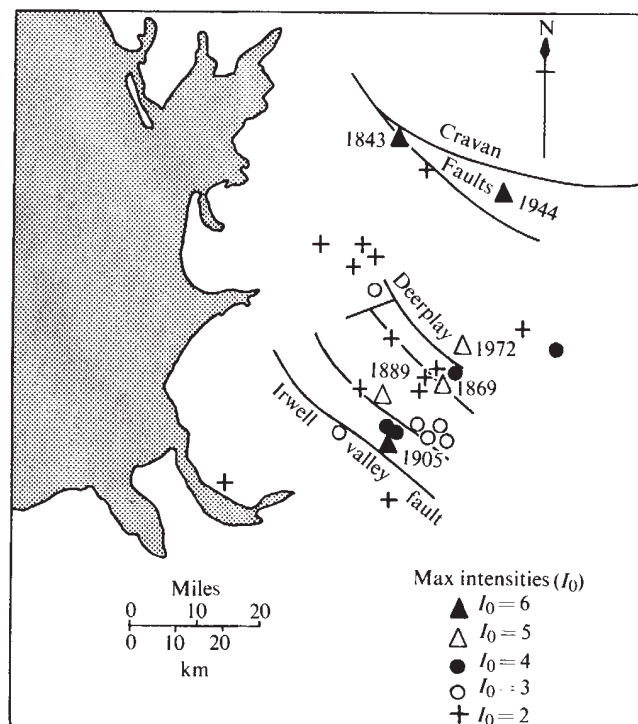
One of the interesting, though not unexpected, features of Davison's list of historic earthquakes is that both the number of events and the proportion of smaller shocks appear to increase significantly with time. However, this is much more likely to reflect the gradually improving

reporting efficiency of an increasing and increasingly educated population than any fundamental change in Britain's seismic characteristics. Only ninety-eight earthquakes were recorded up to the middle of the eighteenth century, and most were reported only very briefly by contemporary or later chroniclers with many other things on their minds. Thus, for example, did Holinshed record that "on the mondaie in the weeke before Easter [1185], chanced a sore earthquake through all parts of this land, such a one as the like had not beene heard of in England sithens the beginning of the world. For stones that laie couched fast in the earth, were remouued out of their places, stone houses were overthrowne, and the great church of Lincolne was rent from the top downwards". From this sort of evidence Davison was sometimes able to assign an approximate intensity (VIII in this case), although it is clear that only the larger of the British earthquakes would ever have been noticed in the first place.

But in 1750 something occurred which could hardly go unnoticed in the increasingly scientific climate of the times and which was to lead to the first serious scientific study of British earthquakes. For in that year, the "year of earthquakes" as William Stukeley called it, no less than four earthquakes of intensity VII took place—two in London, one in Chichester and one in Northampton. One result was that between February 19, when the first London event occurred, and the end of the year about fifty papers on the subject of the 1750 earthquakes were presented before the Royal Society, not to mention several other more general papers inspired by the same events. More importantly perhaps, the interest aroused by the year of earthquakes induced scientists to begin making lists of both British and foreign shocks and thus to pay more attention to earthquake reporting.

The recording of British earthquakes during about the following two centuries left much to be desired, of course, because neither the physical nor mathematical apparatus was adequate for what one would now regard as essential description. The concept of magnitude, for example, was not invented until the 1930s and instrumentation, though improving all the time, has only recently achieved anything like a respectable coverage. (Some would dispute even that.) Nevertheless, the largely non-instrumental techniques using arbitrary (intensity) scales of measurement did allow scientists to construct fairly complete pictures of what are now termed the macroseismic aspects of Britain's larger earthquakes.

The largest intensity (IX) earthquake known in Britain occurred on April 22, 1884, in Colchester and did much to disabuse the Victorians of the idea that British earthquakes are all harmless curiosities. The damage was so great, in fact, that a disaster fund (the first?) was set up to help those owners of the 1,213 buildings needing repair who were unable to bear their own costs. But although the Colchester shock exceeded all others in terms of concentrated severity, the number of towns and villages damaged, the area of structural damage and the total disturbed area were far greater in the Hereford earthquake of December 17, 1896 (intensity VIII). Moreover, the



Location of past events and major faults in the Lancashire region.

residents of Hereford would have had cause to consider themselves unjustly treated, for an event of the same intensity had occurred there only 33 years before. It is certainly a pity that these and many other events were too early to have magnitudes determined because intensity involves so many factors other than seismic energy. Of British earthquakes recorded since magnitude scales were introduced, the strongest ($m_b=4.9$) occurred in the north of England near Kirkby Stephen on August 9, 1970 (see Browning and Jacob, *Nature*, **228**, 835; 1970).

The Todmorden earthquake of March 7, 1972, details of which are reported by Lilwall and Riddle in next Monday's *Nature Physical Science* (August 20), was far from being the largest British earthquake, either in magnitude (4.0) or intensity. But what it does share with other larger and smaller British events is its obscure origin. Very little, if any, detailed work has ever been done on the causes of earthquakes in Britain, but since no plate boundary passes anywhere near the country it may safely be assumed that relatively minor fault slippage is responsible, even though epicentres very often fail to correlate with surface faulting. Little direct evidence is available, but as the accompanying map shows for the Lancashire region, epicentres of the Todmorden and other nearby historic events are clearly related geographically to the faulting in the local Carboniferous rocks. P. J. S.

PROTEINS

Finger on the Pulse

from our Molecular Biology Correspondent
WHETHER the protagonists in this tale of the true nature of protein structure were conscious of the benign shade of Linderström-Lang at their elbow is not recorded in their papers; but it is remarkable how closely the results and even the tools with which they were quarried were prefigured in his work of twenty years ago and more. Now that the ground rules of structure, as seen in static terms, have been laid by the crystallographers, and the thermodynamic basis of conformational stability is in principle established, it is now the dynamic characteristics of globular proteins over which the moving finger is increasingly hovering. The work of Anfinsen and his colleagues, on the interaction of peptide fragments of staphylococcal nuclease to make enzymatically active species, which has already produced so many diverting lessons in

protein chemistry, has also now made possible a pretty demonstration of structural fluctuation, or breathing, in the native conformation.

The nuclease consists of a chain of 149 residues, of which the first five are dispensable, and the remainder can be non-covalently reconstituted by mixing the fragments derived by cleavage at residue 49. The complex has all the conformational and enzymatic characteristics of the pristine nuclease. Taniuchi (*J. biol. Chem.*, **248**, 5164; 1973) has now made such a modified enzyme by mixing the fragment (6-48) with preparations of (50-149), labelled with radioactive acetyl groups at three places in the chain. When this complex was mixed with unlabelled (50-149), it

was possible to observe exchange of the latter with its labelled counterpart in the native conformation, a reaction that can occur only by dint of transient unfolding to break the numerous non-covalent interactions that stabilize the association of the two parts of the chain. Fragment and enzyme are easily separated by affinity chromatography on a column bearing the bound inhibitor, thymidine diphosphate, and with this system exchange of partners can be detected within minutes. There is a strong temperature effect, but even at 4° C the exchange looks to be heading for completion after a few hours. At 15° C, the rate follows a first-order law, though at 4° C there is some deviation at longer times. The activation energy is large,

Ribosomal Genes in Higher Organisms

GENETIC studies on microorganisms have the great advantage that the doubling time is relatively short and this facilitates the production of large masses of selected mutant strains. Mapping genes for ribosomal RNAs and ribosomal proteins, for example, is much more straightforward in *Escherichia coli* than it is at present in the case of higher organisms. Still, some progress may be reported in the case of the fruit fly *Drosophila* on which genetic studies have been carried out since early in this century. The bobbed (bb) bristle phenotype is known to be associated with reduced ribosome synthesis and in *Nature New Biology* next Wednesday (August 22) Steffensen examines the ribosomal proteins of variants of the bobbed phenotype by polyacrylamide gel electrophoresis (unfortunately in only one dimension). He was unable to detect any difference, but his findings do not exclude the possibility that there are differences which might be discovered by using fractionation methods affording higher resolution of the ribosomal proteins. Screening of ribosomal proteins from 80S *D. melanogaster* particles of adult XY males or XO males and XX or XXY also failed to turn up any differences by the same criterion. There were, however, several differences between the ribosomal proteins of *D. melanogaster* and *D. simulans* and their hybrids. Steffensen describes the optimal conditions for producing 60S and 40S subparticles of ribosomes from these species and correlates changes in the electrophoretic patterns of the ribosomal proteins with the genetic constitution.

Steffensen locates seven variants of ribosomal protein genes, three from the 40S, and four from the 60S subunit, in the X chromosome near, and to the left of, the nucleolus organizer. More precisely, it seems that the genes corresponding to the variant proteins may lie in the block between *ot* (outstretched)

and *unc* (uncoordinated) which includes section 19A-E or about twenty-six bands on the map of the salivary X chromosome. Preliminary evidence leads Steffensen further to suspect that segment 19E may be responsible for the variant proteins. The proximity of the ribosomal protein genes to the nucleolus organizer is interesting because the nucleolus organizer contains the DNA which codes for ribosomal RNA. Steffensen predicts that the genes for all the ribosomal proteins in *Drosophila* will be found clustered in the same region near to that of rDNA as indeed has been found to be the case in *E. coli*.

Timmis and Ingle in this same issue of *Nature New Biology* have studied the levels of rDNA in descendants of the plastic genotroph (P1) of flax which produces a large stable form (*L*) or a small stable form (*S*), according to the fertilizers applied. The *L* genotroph contains 16% more DNA than that of *S*, with P1 intermediate. Cytological observations moreover indicate that the nucleolar dry mass in the *L* form is twice as great as that of the *S* form. Comparisons are made with the Mexican axolotl and bobbed mutants of *Drosophila* in which the size of the nucleoli has been directly correlated with the redundancy of rDNA.

Hybridization experiments by Timmis and Ingle confirm their hypothesis that the same type of differences may occur in these examples from the Plant Kingdom. Indeed they find that the percentage hybridization of the *S* DNA was 0.34 and 0.53 with the large and small subunit ribosomal RNAs, respectively, whereas the corresponding figures for the *L* DNA were 0.52 and 0.76, respectively—roughly 70% greater. The total number of genes was calculated in each case: in *S* DNA, 2,095 and 2,055 for the large and small subunit ribosomal RNAs respectively; in *L* DNA, 3,315 and 3,534, for the corresponding RNAs.

and of the order associated with denaturation of proteins. Moreover the rate varies in first-order fashion with the concentration of the complex, and is independent of that of the free fragment, which indicates that the process is limited, as one would suppose, by the unfolding, or breathing frequency, rather than the reassociation rate, which is known from earlier studies to be fast. Now although the stability of the complex is somewhat lower than that of the integral enzyme, as judged by the thermal denaturation temperature, it may reasonably be inferred that the breathing equilibrium obtains in the latter. The cooperativity of the conformational equilibrium ensures that the native state will normally be overwhelmingly populated. Addition of the inhibitor, TDP and calcium ions, causes a dramatic reduction in the exchange rate by stabilizing the native structure.

The dynamic state of the native conformation, and the transient appearance of an unfolded form, is equally reflected in the kinetics of hydrogen exchange, an approach conceived long ago by Linderström-Lang. The internal exchangeable hydrogens in a globular protein evidently become available by this mechanism, and Takesada, Nakanishi and Tsuboi (*J. mol. Biol.*, **77**, 605; 1973) have now extended their earlier analysis, based on the use of infrared spectroscopy to follow the exchange of peptide hydrogens against deuterium, to another protein, α lactalbumin. The supposition is that the unperturbed exchange rate for accessible protons is attenuated by a factor representing the equilibrium concentration of the unfolded form. Now the thermal denaturation profile can be used to yield the enthalpy and entropy of unfolding, and thence to derive the conformational equilibrium constant at temperatures outside the transition range, where the denatured state cannot be detected by spectroscopic methods. For lysozyme this constant leads to an excellent quantitative fit of the hydrogen exchange kinetics. There was also, as in staphylococcal nuclease, a large stabilization of the native state when specific inhibitors were introduced. In α lactalbumin there is a difference between the equilibrium constant for unfolding within the optical transition, and that inferred from hydrogen exchange at lower temperatures, which is most easily explained in terms of the existence of locally unfolded intermediates which govern the exchange rate. The number of slowly exchanging peptide hydrogens is thirty-five in lactalbumin, compared with forty-four in lysozyme, and this difference can be rationalized in terms of differences in the number of inferred intrapeptide hydrogen bonds in the conjectural lactalbumin structure, derived by fitting the sequence to the X-ray model

of lysozyme, to which it is closely related.

Local structural fluctuations also explain new tritium-exchange results of remarkable refinement, obtained by Englander and Rolfe (*J. biol. Chem.*, **248**, 4852; 1973) on liganded and unliganded haemoglobin. This work represents the next stage of evolution of the method, for Englander has found that any transfer of exchangeable protons from a slower to a faster exchanging category, in consequence of a conformational perturbation of the protein, can be observed with great precision after partial tritiation of the protein in one of its two states, by measuring the exchange-out rates, first when it is in the same state, and then when it is in the other. By manipulating these combinations of exchange conditions, it is possible to obtain difference curves for exchange in the two states. Moreover, by choosing the time range (just as in

relaxation experiments), from seconds to days, one can home in at will on the group of protons that show the most interesting differences. In haemoglobin, Englander and Rolfe find that there are three groups of protons, three, eight and nine in number, the exchange of which is ligand-sensitive. All of them exchange much faster in the liganded protein. Inasmuch as the rates are, as before, limited by the equilibrium between folded and (in this case locally) unfolded chain segments, an equilibrium constant and free energy can be attached to the conformational element corresponding to each group. A small energetic change, involving perhaps 2 kcal/mole, is sufficient to give rise to a readily measurable effect on the exchange, such as here observed. The greater local stability of the deoxyhaemoglobin befits what is regarded as the "relaxed" state, the haem ligand perturbing the conformation in the direc-

Immunoglobulin mRNA Sequences

THE exploratory work on messenger RNA sequences was for practical reasons confined to viral RNAs of *Escherichia coli*. The consequent development of highly sophisticated sequencing methods evolved to cope with the problems which arose in this work has opened up the possibility in future of sequencing other nucleic acids. The next obvious stage is to compare the sequences of these viral messengers (which are likely to possess distinctive functional sequences, for example, for replicative purposes) with those of normal cellular messengers, and to compare the sequences of eukaryotic and prokaryotic mRNAs, of both cellular and viral origin. Brownlee *et al.* (see *Nature New Biology* next Wednesday) have made an important step in this direction by determining the sequences of five long stretches within the mRNA chains for mouse immunoglobulin light chains from a myeloma tumour cell line grown in tissue culture.

The advantage of this system over many others from eukaryotes is the possibility of obtaining pure mRNA sufficiently highly labelled with ^{32}P for sequencing operations. Despite lack of mRNA contamination of the relevant 12–14S RNA fraction isolated by sucrose gradient centrifugation, there proved to be contamination with mitochondrial ribosomal RNA in the same size range. Brownlee *et al.* overcame this difficulty by using oligo(dT)cellulose column chromatography, the messenger being more tightly bound by virtue of its poly (A) "tail". Four out of five long sequences (more than eighteen residues) in the purified messenger were found to correspond, with reference to the genetic code, to known amino acid

sequences in the corresponding mouse immunoglobulin light chains. Moreover, the authors were able to deduce from their results the actual triplet codons (where several might be possible owing to the degeneracy of the genetic code) which have been selected for specific amino acids in the immunoglobulin sequence. The nucleotide sequence of a fifth long fragment of the messenger does not correspond to any known amino acid sequence in the light chains and therefore is presumed to be located outside the coding region of the RNA.

The molecular weights of the immunoglobulin light chain mRNA and its 3'-poly (A) "tail", isolated after digestion of the intact mRNA with nucleases, were determined from the electrophoretic mobilities of these species in polyacrylamide gels containing formamide. It is concluded that the total length of the molecule is 1,200 residues and that of the poly (A) "tail" is 200 residues. By comparison, the poly (A) "tails" of HeLa cell mRNA are also about 200 residues long, but those of the mouse globin mRNAs are only 40–80, and that of polio viral RNA is only about 90; histone mRNAs contain none. The coding region must be 690 residues long (3 times the number of amino acids). Subtracting this number plus 200 residues of the poly (A) "tail" from the total length of the molecule leaves 300 extra bases. It is not yet known how these are distributed between the 3' and 5' ends of the mRNA, but it is interesting to note that the 5' terminus of the sequenced viral RNAs (MS2 and R17) and rabbit globin mRNA contains only about 150 extra bases at the 5' end.

tion of higher conformational energy. Reasonable conjectures can then be made from Perutz's structural data about the segments associated with the responsive groups of protons. The method clearly has great potential for wider exploitation, based as it is on the amplification of energetically minuscule events.

DNA SYNTHESIS

Filling the Gaps

from our Molecular Genetics Correspondent

THE idea first proposed by Okazaki that DNA chains are synthesized in short pieces which are only later joined together explains many of the problems of replications, in particular how two chains of opposite polarity are synthesized by an enzyme which moves in only one direction. But no DNA polymerase yet discovered is able, *in vitro* at any rate, to start synthesis of a chain. A solution to the problem of how each Okazaki fragment is started was first proposed by Okazaki and his colleagues last year and is now expanded in more detail by Hirose, Okazaki and Tamanoi (*J. molec. Biol.*, **77**, 501; 1973).

Each fragment of DNA starts off with a length of RNA according to this model; the RNA is removed before synthesis of the fragment is completed. The RNA-DNA nascent fragments can be isolated by their buoyant density, greater than that of single strands of DNA, in CsCl gradients. The proportion of RNA can be estimated from the extent of the buoyant density shift and Hirose *et al.* report that pulse-labelled Okazaki fragments of 400–2,000 nucleotides contain an RNA segment of 50–100 nucleotides. The same length is estimated by measuring the decrease in the length of the fragments when the RNA moiety is degraded by alkali or RNase; and RNA chains of this length can be isolated after degradation of the DNA moiety with DNase.

If the RNA chain initiates each fragment, it must be located at the 5' end. The small shift in size of the fragment when RNA is removed shows that it must be located at one end; labelling the 5' base by removing its phosphate and replacing it with ^{32}P confirms that it is part of RNA. After the RNA has been degraded, similar experiments can be used to label the first base of the remaining DNA chain. The number of 5' RNA and DNA termini labelled in this way is always identical, confirming that one RNA sequence lies at the beginning of each DNA fragment. The first base of the DNA chain is always cytidine and the last base of the RNA is always a pyrimidine, so that it is possible that a specific sequence—presumably repeated very many times in the *Escherichia coli* genome—provides the point of switch from RNA to DNA.

The ineluctable progress of Okazaki's research therefore makes inescapable the conclusion that DNA chains must start with RNA, leaving for resolution the important questions of the sequences (if any) which dictate where RNA synthesis begins and ends and the nature of the RNA polymerase which transcribes them.

While or after the RNA is removed by an unknown mechanism, the 3' end of one Okazaki fragment must be extended to the 5' end of the next fragment. But this leaves a break in the integrity of the phosphodiester chain between the two fragments. The existence and making good of this nick has been one of the more controversial aspects of the Okazaki model for discontinuous growth of DNA chains. The enzyme polynucleotide ligase has been implicated in this action during phage T4 replication, but mutations in the ligase of *E. coli* have produced more equivocal results, taken to cast some doubt on the idea that ligase has this function in bacterial replication.

Mutations in the ligase gene of *E. coli* have now been placed in a standard genetic background by Gottesman, Hicks and Gellert (*ibid.*, 531) and Konrad, Modrich and Lehman (*ibid.*, 519). One ligase mutant, *lig-4*, was originally isolated as a double mutant, *lig-4 lop-8*. The *lig* mutation produces a ligase enzyme defective at high temperature; *lop* increases the amount of enzyme in the cell without altering its sequence and is therefore a control mutation of some kind. Both map close together. Another ligase mutant, *lig ts-7*, was isolated in a genetic background containing many other mutations and has now been separated from them; *lig-4*

has been separated from *lop-8*, so that two strains of ligase mutant are available for analysis.

Both strains have at high temperature a much impaired ability to join together Okazaki fragments and therefore accumulate 10S pieces of DNA. Although the *lig ts-7* mutant is a conditional lethal which dies at high temperature, the *lig-4* mutant can grow in these conditions. The cells of *lig ts-7* are less active in ligase function at high temperature than those of *lig-4*, so that the continued viability of the latter mutant may be due to the persistence of some ligase activity even at high temperature.

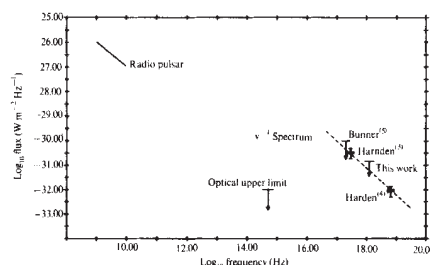
Ligase is clearly identified by these experiments as the enzyme responsible for the final step in joining together the Okazaki fragments. Because double mutants of *polA* and *lig-4* are inviable at high temperature, it is possible that the Kornberg polymerase I may also be involved in this step and perhaps in the removal of RNA and extension of Okazaki fragments until they are adjacent. The *lig-4* cells have only 1% of the wild type ligase activity at high temperature; *lig-4 lop-8* double mutants have about 5%. Both cell types can grow, although defective in sealing Okazaki fragments together; this points to the conclusion that cells contain far more ligase activity than they need, explaining the reason for mutations producing equivocal results when higher levels of ligase activity are possible. It is necessary to achieve a very great reduction in the ligase activity to prevent its action in replication. The purpose of the excess ligase activity is a matter for speculation at present, but it may be implicated in repair and recombination pathways.

X-Ray Flux from the Vela Pulsar

IN next Monday's *Nature Physical Science* (August 20) Cruise and Newton report X-ray observations of the vicinity of PSR0833-45 (the Vela pulsar) and two other pulsars. Because there is a strong dependence of pulsar luminosity on pulsar period, it is not surprising that no X rays were detected from MP0833-40 (period 0.766 s) or from MP0736-40 (period 0.375 s), but they were observed because of their proximity on the sky to the Vela pulsar (period 0.089 s). The observations of the latter, however, help to provide constraints on the nature of the pulsar's spectrum (see figure).

The observations were made using a proportional counter sensitive in the range 2.8 to 9.9 keV, mounted on a Skylark rocket flown from Woomera on November 11, 1970. Together with two positive observations by Harnden (*Astrophys. J. Lett.*, **172**, L91; 1972) and upper limits due to Bunner (*God-*

dard Space Flight Center Report X-683-71-375), the latest upper limits require that the spectrum is either a power law with exponent -1 or an unusual spectrum with a double peak in the X-ray region. A power law spectrum of injected electron energies, with exponent -2 , would produce the required X-ray spectrum, according to Cruise and Newton.



Pulsed energy spectrum of the Vela pulsar, PSR0833-45.

LIQUID HELIUM

Nuclear Cooling

from our Condensed Matter Correspondent

Thermal contact between a metal and liquid ^3He at temperatures of a few mK can be greatly enhanced by adding a small proportion of magnetic impurities to the metal. This important experimental effect has been demonstrated by Avenel, Berglund, Gylling, Phillips and Vetleseter of the Helsinki University of Technology, working in collaboration with Vuorio of the University of Helsinki (*Phys. Rev. Lett.*, **31**, 76; 1973).

Temperatures significantly below 1 mK have been reached only through adiabatic nuclear demagnetization, by which the system of nuclei in, for example, a piece of copper can be cooled to a few μK . Unfortunately, however, this technique has been of little practical use, chiefly because of the difficulty of making satisfactory thermal contact at these temperatures. The need, engendered by the recent discovery of new, perhaps superfluid, phases of ^3He , to carry detailed investigations of the liquid to well below 1 mK will probably be met, eventually, only through use of nuclear cooling, so it has become a matter of prime importance that a method should be devised for achieving adequate thermal contact between metal and liquid at these temperatures.

When heat, in the form of a net flow of phonons (quantized lattice vibrations, travelling at the velocity of sound), passes across a boundary between two different materials, there is always a discontinuity in temperature across the interface as a result of the so-called Kapitza boundary resistance, R_B . This phenomenon, which arises from the different velocities of sound in the two materials, becomes very much more marked as the temperature is reduced (in fact $R_B \propto T^{-3}$), and the discontinuity takes a particularly large value in the case of a helium to metal interface simply because the acoustical velocity mismatch is then especially severe. For this reason, it usually takes an exceedingly long time to establish thermal equilibrium between liquid ^3He and a solid with which it is in thermal contact.

Abel, Anderson, Black and Wheatley, then of the University of Illinois, were therefore surprised to observe that thermal contact between liquid ^3He and the paramagnetic salt cerium magnesium nitrate (CMN) was anomalously good (*Phys. Rev. Lett.*, **16**, 273; 1966). They found, in fact, that the thermal equilibrium time for a mixture of powdered CMN and ^3He was only a few tens of seconds, considerably less than the time predicted from a value of R_B calculated on the basis of phonon conduction. Furthermore, the measured R_B varied

as T^{-1} rather than T^{-3} , which suggested that heat was being transferred across the interface by an entirely different mechanism.

Leggett of the University of Sussex and Vuorio were later able to explain this phenomenon in terms of a direct interaction between the magnetic moments of the ^3He nuclei and the electronic spin magnetic moments of the cerium ions in the CMN (*J. low temp. Phys.*, **3**, 359; 1970). Their proposal was that most of the heat is carried across the interface, not in the form of phonons, but as a result of the electronic spin system in the CMN being able to "talk" directly to the nuclear spin system of the ^3He , and they were able to show that this process would result in a T^{-1} dependence of R_B , as observed. It was suggested, therefore, that, by a similar mechanism, R_B between ^3He and a metal might be reduced if the metal were "doped" with a small proportion of magnetic ions, which could then communicate directly with the ^3He nuclei. The experiment now reported by the Helsinki group

shows unambiguously that this is so.

In one experiment, a thin palladium foil of area 254 cm^2 and containing 32 p.p.m. of iron was immersed in liquid ^3He . The foil was welded to a piece of high purity copper whose temperature, and thus that of the foil itself, could be derived from measurement of its nuclear magnetic susceptibility. The temperature of the ^3He was determined by measuring the magnetic susceptibility of powdered CMN immersed in it. Heat was supplied at a constant rate, and the temperature difference between the foil and the ^3He was carefully monitored as the whole assembly gradually warmed up. R_B was found to vary as T^{-1} , just as for an interface with CMN, and at 4 mK was fifty times smaller than would have been expected for a pure palladium foil, assuming conduction only by phonons.

Although R_B will need to be reduced by an even larger factor before nuclear demagnetization can become a really useful tool for cooling liquid ^3He , the results of the Helsinki experiment are distinctly encouraging.

Subduction Beneath Barbados

ALTHOUGH most of the Atlantic continental margins are not plate boundaries, there are a few border areas where oceanic lithosphere is thought to be descending. One of these lies in the vicinity of the Lesser Antilles island arc where, although crustal structure is known in very general terms, the precise position and nature of the subduction zone have remained unclear. In next Monday's *Nature Physical Science* (August 20), however, Westbrook *et al.* report the results of a geophysical survey which has enabled them to present a more detailed model of the nature and behaviour of the descending crust near Barbados.

The principal data come from an east-west seismic refraction line to the east of Barbados, and show that the Moho dips by 1.3° or 2.0° towards the island (assuming velocities of 8.1 or 8.3 km s^{-1} , respectively, beneath the Moho) and that the oceanic layer dips in the same direction by about 1° . What these results mean is that over 50 km away from, and to the east of, Barbados, at least, there is no significant descent of the oceanic lithosphere. But an interpretation involving a combination of the seismic data, gravity observations and other seismic results from the west of Barbados shows that closer in towards the island the oceanic crust begins to dip much more steeply from both the east and west. At the same time the sediment gets thicker from both directions until beneath Barbados itself it reaches a maximum thickness of about 18 km.

Thus although there is a long gentle slope downwards from the east in the oceanic lithosphere, the subduction zone proper only begins beneath Barbados itself—and this is confirmed by the lack of earthquake activity to the east of the island. Moreover, the sedimentary thickening towards a maximum indicates a sediment-filled trench structure, also beneath the island. Reflexion experiments show that the sediments themselves are deformed, both in the trench structure and over the relatively flat lying oceanic crust to the east. Westbrook and his colleagues attribute this deformation to the piling up of sediment as the Atlantic ocean floor moves westwards beneath. Most of this piling up occurs to the east of Barbados, although the reflexion data also suggest that some sediment is being thrust over the plate to the west of the Barbados Ridge.

Finally, assuming that the natural thickness of the sediment accumulating on the westward-moving oceanic crust has remained roughly constant over the estimated 60 million-year age of the Lesser Antilles, the volume of sediment now in the Barbados pile would imply an average subduction rate of 1.0 cm yr^{-1} . This compares with a figure of 0.52 cm yr^{-1} derived from the fact that the leading edge of the descending lithosphere, assumed to be represented by the lower limit of earthquake foci, has apparently reached a depth of 200 km during the same 60 million years.

COMPACT OBJECTS

Still More Questions than Answers

from a Correspondent

ONE current focus of astronomical interest concerns the existence and properties of compact objects where matter is compressed to an exotic high density state, and where gravity may be so strong that relativistic effects are crucially important. This was the theme of a three-week international meeting on the physics and astrophysics of compact objects held at the Cambridge Institute of Astronomy on July 2–20 and sponsored by the NATO Scientific Affairs Division.

It was of course the discovery of pulsars in 1967 which provided the first strong evidence that neutron stars actually occur in nature. An intensive recent search by astronomers at Jodrell Bank has boosted the number of known pulsars to 100, and the present data were summarized by Professor F. G. Smith. Several theorists—including Professor F. D. Kahn and Dr L. Mestel (University of Manchester), Drs F. C. Michel (Rice University) and R. N. Henriksen (University of Sussex)—discussed the magnetospheres of spinning neutron stars. The complexity of this subject, even in the case when the magnetic and rotation axes are aligned, suggests that it will still be some time before the non-axisymmetric problem—a prerequisite for any real understanding of the origin of the radio pulses themselves—is properly solved. Precise timing data on pulsar periods and their derivatives can in principle tell something about the nature of the electromagnetic torques whereby the rotational kinetic energy is transformed into pulsed radiation and fast particles. The pulsar NP0531 in the Crab Nebula, with the shortest known period, is the most interesting in this respect. Dr E. Groth (Princeton University) presented data suggesting that these torques depend on the 3.5th power of the spin rate. Random jitter superposed on this secular trend is attributed to “micro-starquakes” which cause fluctuations in the moment of inertia of the neutron star.

Within the past two years the Uhuru satellite has revealed a class of X-ray sources which in some ways resemble pulsars. For instance, the X rays from Hercules X-1 pulse with a regular repetition rate of 1.24 s. This source is a member of a binary system, and orbits around its companion every 1.7 d. The latest data on these sources were presented at the meeting by Dr H. Gursky (American Science and Engineering). The consensus among theorists is that Hercules X-1 resembles a pulsar in the sense that rotation of a neutron star provides the “clock”. It differs from a pulsar, however, in that the power radiated comes not from rotational

kinetic energy but from the gravitational energy of material accreted from the companion star. The infalling material is funnelled down the magnetic field lines and impacts on the neutron star surface at $\sim 0.3c$. The kinetic energy liberated heats the magnetic polar caps to temperatures of several keV, so that thermal X rays are emitted.

Detailed models based on this general scheme were presented by Dr F. Lamb (University of Illinois) and by several other participants. The long-term behaviour of Hercules X-1 is very puzzling. In particular, the apparent 35-d period (in which the X rays seem to be completely extinguished for ~ 22 days out of every 35) stimulated several interpretations. Some of these involved the idea that there is some kind of “negative feedback” whereby the pressure of the X-radiation stems the accretion flow; another possibility is that a free precession of the neutron star (analogous to the Earth’s Chandler wobble) is involved. The latter suggestion would be tenable only if the cores of neutron stars could be rigid solids. The possibility that nuclear matter may crystallize at densities $\gtrsim 10^{15}$ g cm $^{-3}$ was discussed by Drs C. Pethick and J. Shaham (University of Illinois) and by Dr S. M. Chitre (NASA). The issue is still controversial, but is important not only for Hercules X-1 but also for the theory of starquakes and “glitches” in pulsars.

The X-ray source Cygnus X-1, which also lies in a binary system, differs from Hercules X-1 in that the X-ray emission flickers rapidly but irregularly. It is widely conjectured that in this system the compact object is not a neutron star but a black hole, and that the X rays come from hot gas swirling down into the hole. Alternative models for Cygnus X-1 cannot yet be ruled out (though some would be excluded if it could be definitely established that the mass exceeded the theoretical upper limit for either neutron stars or white dwarfs). Nevertheless the possibility that some of the most crucial predictions of Einstein’s theory may soon be subject to observational test was sufficiently exciting to guarantee a strong representation of general relativists at the meeting. The key property of black holes, according to general relativity, is that once they have formed they quickly settle down to a stationary state essentially characterized by only two parameters: mass and angular momentum. Thus the “Kerr solution”, once regarded as a special and perhaps atypical case, is now believed to be of paramount importance because it describes the

metric around any black hole. The theorems establishing this result, due to Israel, Carter, Hawking and others, involve high-brow mathematical techniques. Speakers at the meeting, in particular Drs J. Bardeen (Yale University) and K. Thorne (Caltech), addressed themselves primarily, however, to more straightforward questions pertaining to the flow of material in the vicinity of black holes and the characteristics of the emitted radiation. Conceivably the radiation from Cygnus X-1 or some similar source will reveal some pattern which will enable one to diagnose the precise form of the metric. Until this can be done (and at the moment it seems a remote possibility) the existence of these objects certainly cannot be adduced as evidence for general relativity, since most other theories of gravity also predict black holes (in the sense of objects surrounded by a “horizon”, out of which no signal can be transmitted).

Quite apart from their obvious importance to specialists in relativity and the physics of dense matter, the existence of neutron stars and black holes poses the question of how they formed. Drs W. D. Arnett (University of Texas) and J. C. Wheeler (Harvard University) discussed the late stages of stellar evolution and supernova explosions. At present it is uncertain which type of explosion will leave a neutron star and which will leave a black hole. Some supernovae may even disrupt the original star completely, leaving no compact remnant at all. The formation of compact objects in close binary systems is harder to analyse, because of the likelihood that material will be transferred from one component to the other or else lost from the system in the course of the evolution. Why, also, did the supernova explosion not disrupt the binary system? Possible scenarios were presented by Dr E. van den Heuvel (University of Utrecht), Drs J. Faulkner (University of California, Santa Cruz) and G. Bath (University of Oxford) discussed the relation of these X-ray sources to other puzzling phenomena involving close binaries, such as novae and cataclysmic variables.

As was emphasized by Dr S. I. Shapiro (Princeton University) isolated stellar mass black holes are much less readily detectable than those in binary systems, because inflow of interstellar matter would render them only very faint emitters. Black holes of much larger mass in intergalactic space would, however, accrete at a much higher rate and could, according to Dr J. E. Pringle (University of Sussex), perhaps be detected. Dr J. E. Gunn (Caltech) pointed out that such objects would in any case reveal themselves by way of the gravitational lens effect if they were sufficiently numerous.

Many theorists have speculated that the phenomena associated with active

galactic nuclei and quasars may involve a scaled-up version of the same processes as occur in pulsars and compact X-ray sources. Specific ideas along these lines were presented by Drs J. P. Ostriker (Princeton University) and R. D. Blandford (University of Cambridge). But data on these faint extragalactic objects accumulate relatively slowly although remarkably detailed new spectra of some high redshift quasars were reported by Dr P. A. Strittmatter (University of Arizona). There seems much less chance of achieving any real understanding of these extragalactic objects in the near future than in the case of pulsars and X-ray binaries. At the meeting Professor G. Burbidge (University of California, San Diego) reiterated his doubts about whether the quasars are at cosmological distances, and one suspects that this level of basic scepticism may be just as legitimate in five years' time as it is now.

It was perhaps not surprising that this meeting raised more questions than it answered. The nature of these questions is such that their solution demands the collective expertise of physicists and astronomers with a broad range of skills. They also require data, or even co-ordinated observations, spanning the whole accessible electromagnetic spectrum. Studies of these enigmatic compact objects have thus already helped both to stimulate collaboration between astronomers using different observational techniques, and to incorporate astrophysics and relativity more fully in the mainstream of contemporary physics.

NUCLEAR PHYSICS

Potential Ambiguities

from our Nuclear Structure Correspondent
SEVERAL recent investigations have shown that the ambiguities in the optical potentials describing the elastic scattering of helions and alpha particles by nuclei disappear at high enough energies, usually above about 100 MeV. A semi-classical analysis by Goldberg and Smith (*Phys. Rev. Lett.*, **29**, 500; 1972) showed that this can be understood in terms of the maximum (or critical) scattering angle θ_c . The ambiguity is only resolved if the differential cross-section is known for angles greater than θ_c . The critical angle decreases as the energy increases, so that it only becomes possible to resolve the ambiguity above a critical energy, provided the cross-section is measured to large enough angles. The critical angle also increases with the nuclear mass, A , so that it requires a higher energy to resolve the ambiguity for heavy nuclei than it does for light nuclei (see *Nature phys. Sci.*, **239**, 66; 1972).

Further insight into this question has now been obtained by the quantum-mechanical analysis of Goldberg, Smith,

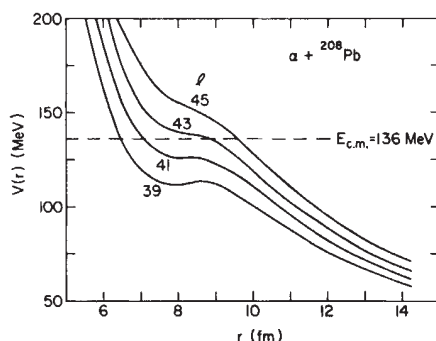


Fig. 1 Effective potentials for the elastic scattering of alpha particles by ^{208}Pb for values of l around 43.

Pugh, Roos and Wall (*Phys. Rev.*, **C7**, 1938; 1973). They examine the behaviour of the phase shifts for each partial wave as the incident energy changes, and find that it changes in character above a critical energy E_c ; at energies less than E_c the ambiguities occur, but for higher energies they are extinguished.

To see how this comes about, recall that the differential cross-section may be expressed as a function of the phase shifts so that ambiguous potentials must give very nearly the same phase shifts for all the partial waves that contribute to the scattering. But a phase shift is an angle, so increasing or decreasing it by any integral multiple of π does not affect the cross-section. Thus if all the phase shifts below a critical value l_c of the orbital angular momentum increase by $n\pi$ the ambiguity can occur.

This can happen for helion and alpha-particle scattering, as shown in Fig. 1. The total effective potential $V(r)$ acting on the incident particle of orbital angular momentum l is the sum of the nuclear potential, the electrostatic or Coulomb potential and the centrifugal potential $l(l+1)/r^2$ and this is shown as a function of r for several values of l for alpha particles on ^{208}Pb . These particles are strongly absorbed, so the scattering is essentially determined by the potential beyond the classical turning point defined by the condition that the incident energy equals $V(r)$. Now if the position of the turning point is regarded as a function of incident energy for different values of l , it can be seen from Fig. 1 that it moves inward suddenly at an energy corresponding to the triple point at which the minimum in $V_l(r)$ disappears. This sudden movement of the classical turning point at a critical value of l allows the phase shift to increase by $n\pi$ and hence gives rise to potential ambiguities.

The connexion between this sudden

movement and the ambiguities is shown in Fig. 2. Here several different potentials all giving essentially the same differential cross-section are plotted as a function of radial distance for the critical partial wave with $l=43$. For each potential, the turning point moves in suddenly by amounts corresponding to phase shift increases of π , 2π and 3π . The ambiguities persist for all values of $l < l_c$, but at energies higher than E_c they do not arise, because there is no sudden dip in the effective potential and hence no sudden inward movement of the turning point. For energies less than E_c the ambiguities therefore occur in all partial waves, and thus in the potential as a whole, whereas for energies greater than E_c they do not occur in the higher partial waves. It is not possible in the latter case to find a potential that reproduces both the smooth variation for the higher partial waves and the $n\pi$ increase in the phase shifts for the lower partial waves, and so the potential is unique. It is thus

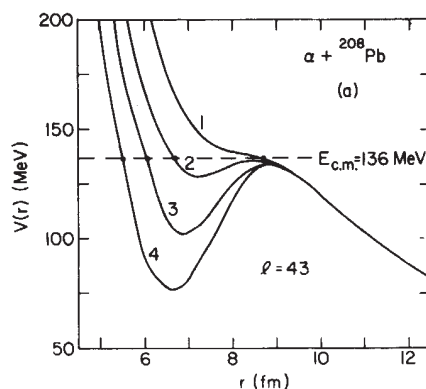


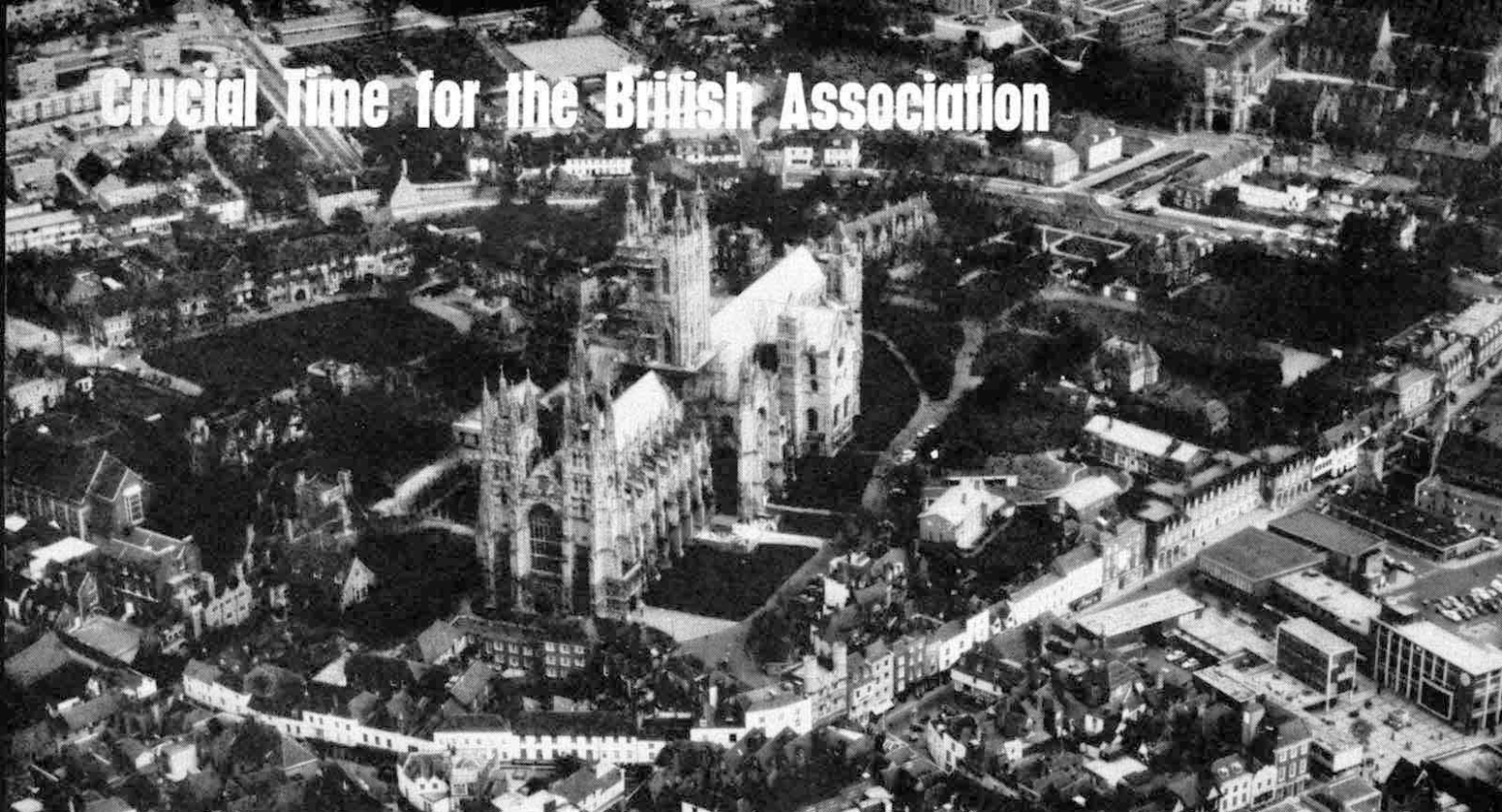
Fig. 2 Effective potentials for the elastic scattering of alpha particles by ^{208}Pb for $l=43$ only. Each of these potentials gives the same scattering, showing that the phase shifts differ by $n\pi$.

possible to understand why the potentials show ambiguities at low energies but not at high energies, and to relate the critical energy to the form of the effective potential.

Correction

IN the News and Views article "Receptor Function" (*Nature*, **244**, 253; 1973), the penultimate sentence in the third paragraph should read "Moreover, during successive ACh applications, the toxin becomes completely effective only after the first contact with ACh, suggesting that its action is dependent on an initial binding of ACh at the receptor." In the published version the word "effective" unfortunately appeared as "ineffective".

Crucial Time for the British Association



Canterbury [Aerofilms Ltd]

THE British Association for the Advancement of Science is breaking new ground once again at its 135th Annual Meeting which is to be held at the University of Kent at Canterbury next week. Through no fault of its own the association is holding its annual meeting in mid-August instead of the traditional time of early September, which might well put back its plans to increase the popularity of the meeting. And it is only a year since the association, in an attempt to shake off its rather lacklustre image, made another break with the past when it decided to compress the annual meeting into a week beginning on Monday instead of spreading the delights over the best part of two weeks with a relaxing weekend in between.

These outward signs that the association is serious about coming to terms with the 1970s do not constitute the entire story, for the most significant changes have been taking place behind the scenes. Since the annual meeting at Leicester last year the Privy Council has approved drastic changes in the association's constitution which, for one thing, will turn the gargantuan general committee into a more manageable body of close on sixty members, more than half of them being elected. This committee will have the powers to elect a council of twenty-two members which will have as its chairman, for the first time, a paid officer of the association. These changes will be instituted, not before their time, after the annual meeting next week and there is little doubt that the association from now on will be a much more wieldy body that should be a better barometer of thoughts and concern in Britain about science and its implications.

As luck has it, the streamlined BA is now in a good position to fight off the challenge, and that is what it is, from the newly formed Council for Science and Society which was set up with generous support from the Leverhulme Trust (see *Nature*, 244, 191; 1973). The stated aim of this council is to identify areas of research with important social consequences and to study these objectively. The comparison of this new council with the much criticized British Society for Social Responsibility in Science is clear, but there are also obvious clashes between its aims and those of the BA. The Royal Society grant to the BA—which amounts to £20,000 a year—is ostensibly for the purpose of “the popularization of science and the conducting of a responsible public

debate on the social consequences of science”. And it must be asked whether, in the long run, this competition—if it will develop—will do the BA more good than harm. This is the question which will be at the front of the minds of delegates to the conference this week. The BA has a head start on the Council for Science and Society and the first reports of the association's investigations, instigated in 1972, to look into matters of scientific concern should be available early in the new year. It is, however, essential that the momentum which has been generated by these investigations into the social consequences of research in biology and genetics and into the place of research in higher education in Britain should be maintained by other studies, and it is significant that the association has now put the responsibility of choosing topics for investigation on to its executive committee.

But the BA is best known for its annual meeting which has been held during all but six of its 141 years of existence. Last year the meeting, to be blunt, attracted rather less support than anyone would wish and it remains to be seen whether the enforced change of date to the middle of the holiday season will boost attendance. The association, after a great deal of soul searching, has decided to persevere with the annual meeting, a decision to be admired, but it is to be hoped that as time goes on that the meeting becomes less of the centre of the universe as far as the association and its members are concerned and that other activities will come into their own and receive due attention.

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Good Times Just Around the Corner for Nuclear Power?

by our Special Correspondent

Nuclear power may finally be about to come into its own. This article examines the past and glances at the future of the industry and the United Kingdom Atomic Energy Authority.

"WITHIN ten to fifteen years we may have reached the point at which every new power station will be a nuclear power station". Those words were not uttered this year. Or last. Indeed, they are a prophecy that was so far from being true that fifteen years after they were said, the nuclear industry in Britain was entering one of the leanest periods in its chequered history.

These words in fact are Sir Edwin Plowden's, the first chairman of the UKAEA. And they were spoken in 1956, the year Calder Hall, the world's first nuclear power station, opened. But in spite of the fact that the targets set in the 1950s and 1960s in Britain's first and second nuclear power programme have not been met, future prospects are now much brighter. It is now likely that in fifteen years time almost every power station ordered will be nuclear.

In the years in between Sir Edwin's statement and now, the history of atomic energy in Britain has not been an entirely happy one. There have indeed been times when the critics of the nuclear industry and the Atomic Energy Authority in Parliament, the press and industry, have seemed with ill-disguised glee to be siding with Noël Coward's three equable businessmen in cheerfully declaring that there are bad times just around the corner.

Why has it taken so long for nuclear power to be on the verge of dominating future orders? And indeed, what part has the UKAEA—the source of those optimistic forecasts of the fifties—played and still got to play in nuclear power? Controlled nuclear energy had its origins on December 2, 1942, in Chicago. On that day the first uranium-graphite pile was operated and man had initiated a self-maintaining nuclear reaction for the first time. Less than fourteen years after that Calder Hall was feeding electricity into the national grid in Britain. In between the Atomic Energy Project and then the UKAEA were set up.

The prime mover was defence. Britain wanted nuclear weapons and in 1946 Harwell was established and the British scientists who had been in North America during the war, helping in the work that led to Hiroshima and Nagasaki, came home. Air-cooled graphite-moderate piles were built at Windscale to produce plutonium, Aldermaston did its calculations, and in October 1952 the Monte Bello test was fired.

But while controlled atomic energy was born in war the possibilities of power generation were realized before then. By 1949, however, it was thought that the only worthwhile early application of atomic energy would be submarine propulsion. Electricity generation using enriched fuel, it was agreed at the time, would not be economic. But in 1950 the possibility of using natural uranium fuel to produce both power and plutonium was realized, and in January 1951 a design study started. The first results were presented at the Harwell Power Conference of 1951 and were heavily criticized. The size and cost of the proposed reactor were both attacked, the advantages of sodium cooling over gas cooling were emphasized, and the Risley team

pressed for all the effort to be put into fast reactors. Incredibly, looking back, the natural-uranium reactor was dropped from the first draft of the 1952 Harwell programme. If that decision had been upheld the face of nuclear power in Britain today would have been radically different. As it was, the design that was to become Calder Hall was rescued by Mr B. L. Goodlett, deputy chief engineer at Harwell, and Mr R. V. Moore, now managing director of the Reactor Group.

In Richard Moore's words, "By the end of 1952 it was certain that a design had been produced which could and should be built". In February 1953 approval was given for the construction of Calder Hall, a Magnox type reactor. British industry—under the authority's management—demonstrating what can be done when the pressure is really on, built the first half of the station in 33.5 months, completing, the second half 6 months later. The end result was primarily a factory to produce plutonium, with power also available as a byproduct.

But while Calder Hall was being completed the first nuclear power programme was announced in 1955. Twelve power stations with a total output of between 1,500 MW and 2,000 MW were to be built in the ten years to 1965. In 1956 the Suez crisis occurred, and the next year the Government reacted to the possibility of Britain being cut off from its oil supply by trebling the nuclear programme. In all, between 5,000 and 6,000 MW were to be installed by 1965. But in October of 1957 the expansion had to be ignominiously rephased as part of restrictions of capital spending and the target date was extended to 1966. In 1960 it was further extended to provide for 5,000 MW by 1968. In fact, excluding Calder Hall and Chapelcross, reactors originally built for plutonium production, Britain managed to achieve only 4,856 MW of installed capacity by August 1971 when Wylfa, the ninth nuclear power station to be built, came on full load. To add to the confusion of planned, rephased and postponed programmes, the second nuclear power programme which was announced in 1964 was intended to provide an additional 5,000 MW of nuclear capacity using advanced gas cooled reactors between 1970 and 1975. This target was raised to 8,000 MW in October 1965, and "for planning purposes" it was assumed that on average over the six years 1970 to 1975 one nuclear power station would be commissioned every year. So far, none of these stations is operative. It is a fairly depressing story. And looking back the mistakes loom large. The panic expansion of the programme in 1957, followed by the stop, go and rephase policies of government, produced a degree of disruption that few industries could cope with, and certainly no industry as poorly put together as the nuclear one. Its shape was wrong from the start. In 1954 no less than four industrial consortia were formed to design and build nuclear power stations, and in 1956 a fifth consortium was added. Despite the steady pruning of the consortia—forced largely by commercial necessity rather than by the desire of the CEBG to deal with only one consortium—the result has been that almost every nuclear power station has been, in effect, a prototype. Hunterston B, the AGR station being constructed in Scotland, is the first repeat order for a nuclear power station. All the earlier stations were significant modifications of their forerunners. Many of the problems that new stations have run into should hardly come as a surprise. Added to this are the technical problems—particularly the steel corrosion problem which has cut the output from some of the Magnox stations by almost 20%.

The other fundamental error lay in ordering the AGRs more or less from the drawing board. The Windscale Advanced Gas Cooled Reactor—which celebrated its tenth anniversary of successful operation earlier this year—is not a full prototype, being designed largely to develop materials and fuel for the commercial stations. The result was three prototype AGRs being built at Dungeness, Hartlepool and Hinckley by three different consortia with all the attendant problems. The current contraction of the industry into just one consortium—which even the Select Committee on Science and Technology saw the need for in 1967—is a much needed remedy. But while the nuclear industry has been forming and reforming itself and while its power stations fell behind schedule and went over budget, what has the UKAEA been up to?

The authority has changed greatly in its eighteen year life. Defence brought it into being, and defence preoccupied it to a greater or lesser extent for its first ten years. Production of plutonium for nuclear weapons led to the building of the “atomic factories” at Windscale, Springfield and Capenhurst and to the large staff of the authority. Once Calder Hall became a reality the UKAEA had the job of underpinning the design and construction of Magnox reactors, followed shortly by the development of other reactor systems. The Dounreay Fast Reactor, the Advanced Gas Cooled Reactor, the Steam Generating Heavy Water Reactor and the High Temperature Reactor were all studied and developed, and the UKAEA’s expenditure and manpower rose rapidly to a peak in 1961–62 (see Fig. 1). But by the mid-1960s, questions were being asked. Between £750 and £800 million of taxpayers’ money had been spent by the authority (some of it on defence work) and all Britain had to show for it, the argument ran, was three working power stations and six more under construction.

Further, it was becoming increasingly clear that much of Harwell’s work on neutron physics and other applied research on the basic aspects of reactors was, if not nearing completion, at least falling in scale. Yet the authority contained a great deal of expertise in many fields that simply could not be disbanded. An added complication was that the production side of the authority, having produced as much plutonium as Britain’s navy and air force could hope to deliver in the event of war, had turned itself into a highly effective fuel organization which was making money—an aspect of the authority’s work that a select committee of Parliament felt was rather at odds with its research and development role.

The result was the Science and Technology Act of 1965 which freed the authority to undertake non-nuclear work and which set up the Trading Fund, a commercial profit and loss account that charted the progress of the fuel and radiochemical sales of the UKAEA.

The changes were important. In 1971 the trading fund was taken to its logical conclusion and British Nuclear Fuels and The Radiochemical Centre were formed as separate companies.

To prove its value to the country and to help offset the cost of running the authority, the AEA has put considerable effort into its contract research, and in 1971–72 its income from non-nuclear and nuclear contracts totalled about £8.5 million. Harwell, for example, now has its work divided up into four roughly equal parts—studies of specific problems on reactor development, examination of long term scientific problems, contract work for industry and contract work for government departments, while Risley earned £3.75 million in foreign currency for contract work last year.

While this side of the authority’s activities has grown, the overall size of the organization has fallen steadily, and the reorganization of the fuel and radiochemical side, allied with transfer of Aldermaston to the Ministry of Defence in April this year, has left the authority—on paper at least—

a more rational organization than it appeared to be a few years ago.

But what of the future? In spite of the black facts of Britain’s nuclear past, the signs are now that nuclear power may be about to meet the promises of the fifties. And for reasons similar to those that set the first nuclear power programme under way. The price of oil—already at close to \$3 a barrel and likely to rise further now that the United States can no longer meet its own needs—augurs well for a future that will have to be largely nuclear. Coal, while plentiful, is unlikely in the immediate future to be minable at a low enough price and in sufficient quantities to meet the growth in energy demands. It is not insignificant that the cheapest electricity generated last year by the CEBG came from some of the nuclear stations. It is equally possible that nuclear stations may prove to be longer lived than the twenty year design life given to Magnox stations.

And once the government has announced which type of thermal reactor is to be built next—and the indications are that it may be another AGR—industry should be able to drag itself out of a three year doldrum in which it has not had a new nuclear order. The long period of stagnation has done no good, but now that the industry is organized into one company—the National Nuclear Corporation—the future has to be brighter.

A new station will be ordered next year, followed by perhaps a further half dozen stations during the 1970s, with orders coming at the rate of three to four a year in the 1980s if the economy behaves itself and follows its predicted path. The first fast breeder order is theoretically due next year. In fact it is unlikely that an order will be placed until the prototype fast reactor at Dounreay, which is currently being commissioned, has proved itself.

There are also indications that we may have learnt from past mistakes. One of the chief problems with the Magnox stations was transferring the technology to industry. With the fast breeder The Nuclear Power Group has been involved in the design and development work since 1969, and, until the reorganization of the industry was announced last year, it was the group that was to build the first commercial fast reactor. The new National Nuclear Corporation will now do that, but doubtless with TNPG’s staff. It is also intended to build just one commercial fast reactor design to start with, avoiding the old problems of too many prototypes. Equally, some of the lessons from the AGR programmes have been learnt and the Dounreay PFR has a number of features—for example, the size of the fuel rods—which could be used in a 1,000 MW commercial station as well as in the 250 MW prototype.

The other key lesson that must be learnt is that industry must be able to build the stations to time and budget if nuclear power is really to pay. The first nuclear programme was planned to cost £300 million and in fact cost £750 million. The CEBG has come tacitly to accept that it takes up to seven years to build a station, and the overruns on the AGRs are horrifying—the cost to the CEBG being enormous. The Japanese company Mitsubishi is said to offer to construct nuclear stations in forty-eight months. While that figure may not be ideal—it is more important that stations are built late and safe than early and sorry—it is still a level of competition worth bearing in mind if Britain’s nuclear company is to be a commercial success in foreign markets as well as at home.

But once the new thermal reactor has been chosen, and once the fast breeder is being built by industry—and the design for a commercial station got under way while the prototype was still being built—what will there be for the UKAEA to do? A favourite argument has been that industry will simply have taken over. The authority will have to be disbanded. But many arguments militate against this.

In the short term there is a lot of work to do. Whichever

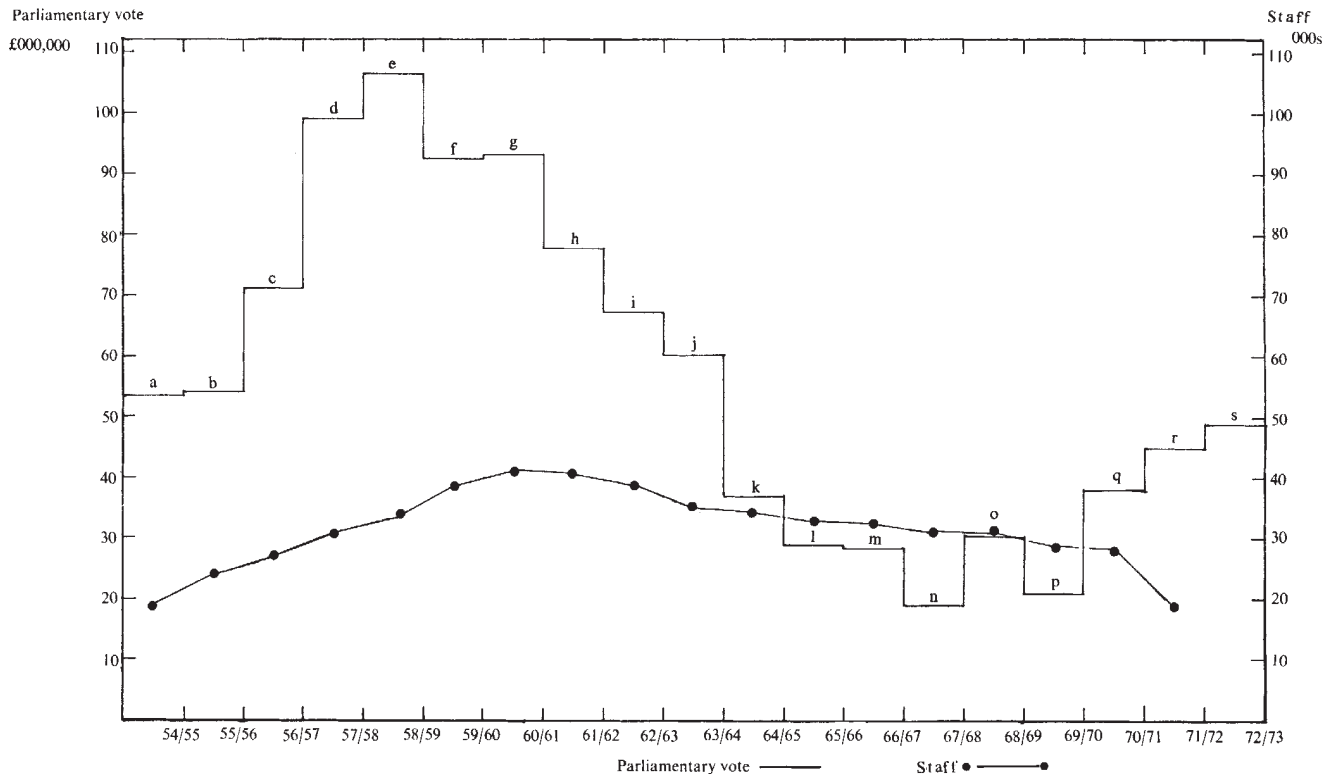


Fig. 1 The UKAEA: the cost to the country*

(a) Work on Calder Hall and Dounreay Fast Reactor already under way. Dounreay started March 1955, Calder Hall March 1953. First Nuclear Power Programme announced February 1955: twelve stations in ten years for £300 million. Four consortia formed. Design of Zeta begins. (b) June 1955 six more reactors for military plutonium production approved. Two more reactors for military plutonium production approved. Two more for Calder Hall, four at Chapelcross. (c) October 1956 Calder Hall opened. Work on Berkley and Bradwell power stations starts in January 1947. A fifth nuclear consortia formed and the revised nuclear programme (5–6,000 MW by 1965), announced. Development of reactors for submarine propulsion begins. February 1957, National Institute for Research in Nuclear Science established. (d) Britain's first megaton range nuclear weapon exploded at Christmas Island, May 1957. August 1957, Zeta on full power. Winfrith establishment set up. Construction of 7 GeV proton synchrotron begins at Rutherford High Energy Laboratory. International Atomic Energy Agency established. Construction of Hinckley Point A and Hunterston A begins. Windscale accident releases radioactivity to atmosphere, October 1957. (e) Construction of Windscale prototype AGR begins. First Chapelcross reactor begins feeding electricity to grid, February 1959, last Calder Hall reactor critical, December 1958. Springfields manufactures its millionth fuel rod. Twelve European countries sign agreement to develop Dragon High Temperature Reactor. Dounreay fast reactor completed. United States pressurized water reactor bought to power Dreadnought submarine. (f) Construction of Trawsfynydd power station begins. Major defence projects completed and all main defence plant commissioned. Capenhurst diffusion plant extensions completed and DFR on power. (g) Culham laboratories established. June 1960, nuclear power programme timetable extended. August 1960, Dungeness A under construction, April 1961, Sizewell B started. Second nuclear submarine ordered. Five consortia reduced to three by mergers. (h) Windscale AGR completed. First Nevada underground test, March 1962. Oldbury A power station construction started. (i) Berkley and Bradwell Magnox stations on load. Latina nuclear power station in Italy—designed by The Nuclear Power Group—on load. Windscale AGR begins generating electricity. Construction of SGHWR begins. Second Nevada test. Fall in enriched uranium requirements for defence leads to 50% cut in Capenhurst staff. Nuclear marine propulsion being studied with view to building prototype ship. (j) Production of U-235 for military purposes stopped. Greater part of capital works programme complete. Wylfa power station construction begins October 1963. Dragon goes critical. (k) Hunterston A, Hinckley Point A and Trawsfynydd on load. Second Nuclear Power Programme announced (AGRs). Millionth magnox fuel element produced at Springfields. Science and Technology Act provides UKAEA freedom to undertake non-nuclear work. Trading Fund set up to run commercial fuel and radiochemical work as self-financing account. National Institute for Research in Nuclear Science transferred to the Science Research Council. Marked drop in grant due to NIRNS transfer, trading fund, and to Ministry of Defence providing eight annual payments to cover capital liabilities outstanding on military plants and the terminal expenses of closing down production (see i and j). First payment £37.7 million. (l) Dungeness B ordered. Fuel policy white paper foresees one nuclear station a year to be commissioned between 1970 and 1975. Capenhurst modernized to provide AGR fuel. Construction of Prototype Fast Reactor approved. Marine propulsion reactors rejected for immediate development, but studies to continue. Underground test at Nevada. Authority unsuccessfully bid to build SGHWRs for two Finnish utilities. (m) British Nuclear Export Executive formed to sell reactors overseas. Sales promotion in Belgium, West Germany, Rumania, Austria, Switzerland, Greece, Finland, Japan. (n) SGHWR completed. PFT construction under way. Hinckley Point B and Hunterston B ordered. Work on fusion to be cut 10% per annum for five years. Tokai Mura Magnox Station in Japan, built by GEC-Simon Carves, on load. (o) Consortia cut to two—British Nuclear Design and Construction and The Nuclear Power Group. TNPG brought in to work on PFR. Forty authority staff transferred to the consortium and 100 others seconded. PFR programme put back a year due to difficulties in manufacturing radiation shield roof. (p) Legislation to form British Nuclear Fuel and the Radiochemical Centre Ltd prepared, but not passed as Parliament dissolved in 1970. Green Paper proposes a British Research and Development Corporation intended to take over staff of research and reactor groups together with five MinTech establishments and the National Research and Development Corporation. Urenco and Centec set up to build gas centrifuge enrichment plant at Capenhurst and Almelo. British Nuclear Export Executive, no reactor sales to its name, disbanded. Culham staff in USSR for fusion experiments on Tokomak. (q) BNFL and Radiochemical Centre Ltd established. Desalination plant at Ipswich approved. National Radiological Protection Board set up and authority Safety and Reliability Directorate formed. Thermal Reactor Review under Peter Vinter examines choice of next reactor type. Heysham AGR ordered. (r) Period of animated suspension while government ponders the future, the authority develops all its reactor types and the industry looks at its emptying order books. Ipswich desalination plant abandoned. (s) Formation of one nuclear company announced. Further eighteen months of studies before decision on next reactor type to be taken. Fusion research stepped up.

* Source: UKAEA annual reports; reporting years run April 1 to March 31.

thermal reactor type is chosen, the UKAEA will have to underpin the development of it by industry. Should light water reactors be chosen there will be at least two or three years work to be done on design before a station can be built—and the authority will certainly have plenty to do on the thorny question of safety. Work on the High Temperature Reactor will undoubtedly continue and the authority should be able to redeploy its resources, which, it maintains, are being stretched by the need to keep so many reactor types under detailed study, into whichever reactor type is chosen. And there is still a lot of underlying work to be done on the fast breeder—perhaps very different from the neutron physics work of the early days, but work on high temperature steels and other work fundamental to component development.

In the longer term lies fusion. Expenditure and effort on fusion work has just been stepped up again and the UKAEA is preparing a cooperative programme with Europe. Fusion has had a tough time in Britain—suffering most from the extreme and, it proved, unfounded optimism that accompanied Zeta's early results in the late 1950s. In 1967 it was decided to cut expenditure by a half over a five year period and disillusion with the hopes of fusion had really set in. Culham entered into the contract research business; but this year's decision to increase expenditure and to design a large experiment has put the impetus back into fusion. A rough timescale for the development of fusion could be that a large experiment to investigate plasma physics will be built for 1978, followed by a larger machine to study a plasma sustained by thermonuclear reactions and refuelled with cold fuel, a system that could perhaps be followed by a prototype generating plant. Such a timescale

—if all goes well and controlled fusion in fact proves possible—could produce fusion reactors by about 2000.

The longer term may also include the development of other reactor types. A glance at history suggests that it would be foolish to assume that the sodium cooled fast breeder, promising though it now looks, is the ultimate in fission reactors. The gas cooled fast breeder is just one other possibility on the horizon.

Most importantly though, the authority will still need to do the fundamental safety work on all reactor systems. When the decision is taken to build a reactor all the questions about its safety have not been answered for all time. The possibility of a nuclear power station accident is not to be treated lightly, and it is fundamentally important that the assumptions on which the proclaimed safety of nuclear power stations is based are continually re-examined.

But safety research does not show a profit, and it cannot easily be left to the nuclear company—particularly if the decision is taken to develop High Temperature Reactors internationally and in industry rather than in the relevant government undertakings—to fully question the safety of its nuclear plant. The need for a body to undertake such research—and studies on the long term reliability of nuclear stations which involve huge capital sums—combined with the need for fuel and waste disposal research guarantees the authority a role. It will be a much changed role from the days when the authority handed down the nuclear lore to British industry. Indeed it may largely consist of acting as a contract research organization for the industry and the generating boards, but it could be a role that will do much to ensure that Britain really does profit from nuclear power.

Whither Climate Now?

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Since Professor Lamb wrote on "The New Look of Climatology" (*Nature*, 223, 1209; 1969) he has set up a research unit in the University of East Anglia for study of the long-term behaviour and current trends of climate.

SURFACE air temperature averaged over the globe seems (Fig. 1) to have been falling since the 1940s, at least up to 1969. Temperatures at lowland sites typical of central England, averaged over the year, also fell successively from the peak value of 9.7°C in the 1940s to 9.5°C in the 1950s and 9.3°C in the 1960s, thereby returning to the average level of 1910–30 or, for that matter, to the average for the hundred years 1851–1950. In the Arctic the recent downturn has been far greater (as was the warming in the 1920s and 1930s) and remarkably abrupt. At the observation post in Franz Josefs Land (80° 20' N, 52° 48' E), which was for long the station nearest the North Pole, ten-year average temperatures from the beginning of the permanent record in the late 1920s up to 1960 varied between –10.8 and –10.0°C; for 1960–69 the figure was –14.5°C.

Figure 2, using a crude but readily available measure of the temperatures prevailing over the main ice-producing areas of the Arctic Ocean, indicates that the lower temperatures which set in abruptly about 1961 in that region have been continuing right up to now despite the fluctuation which has given three mild winters in England since 1970.

A survey covering as much of the northern hemisphere as could be analysed from 1881 to 1960 for the incidence of extreme warm and cold months², with mean temperature departing by $\geq 10^\circ\text{C}$ from the overall average for the same months (all in the cooler seasons of the year), showed the extreme positive departures concentrated in the higher latitudes, particularly over Siberia north of the Arctic circle and over west Greenland (in each of which areas there were ten extreme warm cases in the eighty years and no extreme cold cases, except east of 140° E).

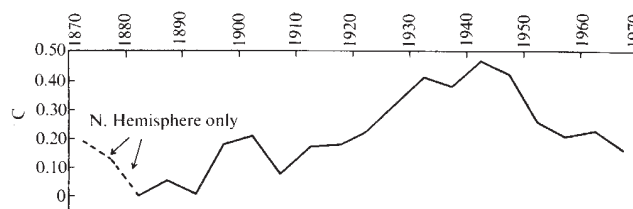


Fig. 1 Changes in global mean surface temperature (five-year averages from 1880–84 to 1965–69, after J. M. Mitchell and NCAR). ---, Northern hemisphere only.

This distribution was evidently associated with the warming of the Arctic and recession of the sea ice which was proceeding most rapidly in the 1920s and 1930s. The extreme cold months in those early years were concentrated in the central regions of the continents of Asia and North America between latitudes 40° N and 65° N. But since 1960 there have been many of

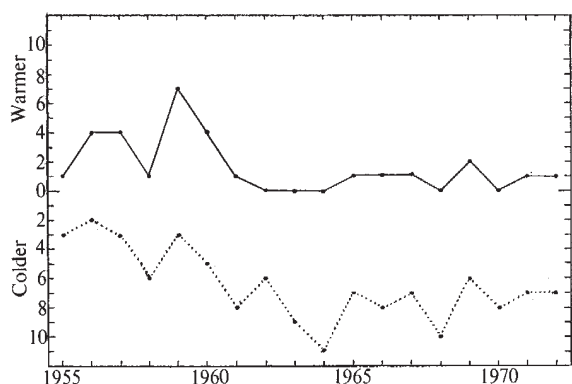


Fig. 2 Numbers of months each year from 1955 to 1972 in which most of the region north of 70° N appeared warmer (—) or colder (....) than the 1931-60 average. Data compiled from the monthly maps of surface temperature anomaly published in ref. 1.

these anomalously cold months in the Franz Josephs Land sector of the Arctic, the extreme case (January 1964) at that point having a mean temperature 18° C below the 1931-60 average.

An important feature of this climatic change seems to have been a change in the general atmospheric circulation. Before 1960 a circumpolar vortex with at most two persistent cold troughs in high latitudes, generally over northeast Canada and northeast Siberia, was characteristic. Since then the monthly patterns have rather frequently shown three troughs, the extra trough being mostly in the Franz Josephs Land-Novaya Zemlya sector. Its introduction has meant greater amplitude and shorter wavelength in the meandering flow of the upper westerlies and, presumably, some net expansion of the cold area of the polar cap bounded by the strong flow of the upper winds. The greater northward and southward excursions of the surface and upper winds over middle latitudes and the sub-Arctic fringe that go with such patterns have produced some extreme warm as well as extreme cold months in latitudes between 40° N and 70° N and an abnormal incidence of blocking anticyclones.

The decline of the westerlies in Britain (Fig. 3), the extreme warmth and dryness of summer 1972 in European Russia with a three-month mean temperature nearly 5° C above the 1931-60 average near the Finnish border, and the mild dry December and January 1972-73 in Scandinavia and central Europe (with little snow at many winter sports resorts and roses flowering in late January in Copenhagen) all fit into this pattern. The same month of January 1973 saw the Labrador ice belt extend beyond its previous record east of Newfoundland and equalled the lowest temperatures observed in the past 140 years at Sverdlovsk in the Urals at 60° E. No doubt this type of behaviour of the large scale circulation of the atmosphere underlies the fact that twelve of the fourteen warmest Januarys in England since 1680 fell within four years of a January with mean temperature below 2° C, six of them within either one or two years of such an opposite extreme. This is also bound up

with a serious difficulty that attends many of the rules derived by statistical significance tests for long-range weather forecasting: the same conditions which precede severe winter in Europe also show a certain enhancement of the frequency of abnormal winter warmth, usually attributable to the sector affected by southerly winds on the western side of a persistent blocking anticyclone.

The increased extent of the Arctic sea ice since 1961 caused understandable alarm in Iceland, which was half surrounded for a time in 1968. But in the past two years the excess ice has been concentrated west of Greenland and in the Canadian sector; the east Greenland ice belt has narrowed again but has consisted of heavy "polar" ice.

These variations have excited interest in the popular press as well as in scientific circles, but particularly in those regions which at any given time have happened to be most adversely affected. With three mild winters in a row since 1970, the longest run of mild winters since 1948-50 in Britain and much of northern Europe (though, at least in England, not overall as mild as those of twenty-three years ago), concern over the trend of our climate seems to have died down. And it may be partly for that reason that the newly established Climatic Research Unit in the School of Environmental Sciences at the University of East Anglia is seriously short of the financial support needed for its continuance.

Nevertheless, the instability of the climatic regime and the possible threats to it from side-effects of man's activities^{3,4} are more widely appreciated than before. And it is safe to predict that the next season of adverse weather will bring a clamour of inquiries and requests for climatic forecasts that we should prepare to meet.

The working group on climatic fluctuations set up by the World Meteorological Organization in 1969, with myself as chairman, had as the main items on its agenda to consider the causes, both natural and man-made, of climatic changes, the demand for advice on future climate and the ability of science to deal with this demand.

The demand confronts meteorological services in all the advanced countries, and many others besides, with a problem that the science is admittedly ill-equipped to meet. It has arisen from the needs of practical decision-making in connexions as various as the provision of adequate supplies of electricity and fuel oil, the incidence of drought in arid lands with rising populations and increasing industrial use of water, shipping insurance against hurricane damage, the future of the Atlantic and Arctic fisheries, forest planning at the northern limit in Finland and in the Solomon Islands where the threat is from tropical cyclones. All these have been affected by the climatic trend since 1950, and in several connexions vulnerability is increased by man's systematic exploitation of the natural resources (particularly water) of the area concerned to the limit.

To develop a scientific approach to the problem of prediction—or, more modestly and realistically stated, of advising on future climatic probabilities—the first essentials clearly are: (i) to establish the facts of the past climatic record in as much detail, over as much of the Earth and as far back in

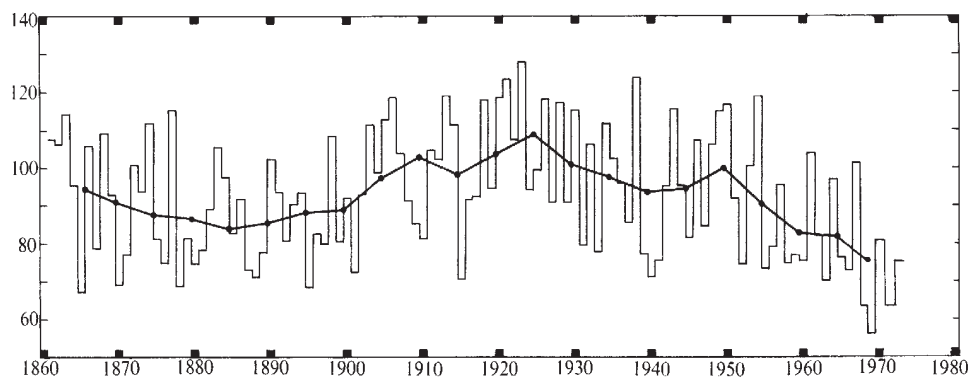


Fig. 3 Number of days per year of general westerly type over the British Isles (1861 to 1972). —, Ten-year means, plotted at five-year intervals (and the latest ten-year mean 1963-72).

time, as the data permit, and (ii) to use this observational record to diagnose the processes at work in bringing about climatic changes. If we are to identify any recurring processes which take many years, and in some cases centuries, for their completion, it is axiomatic that we need the longest possible observational record.

Identification of the circulation processes in the atmosphere and oceans, and of any cyclic changes in the water stored as ice and snow on land and in the soil, as well as of any other relevant physical changes (volcanic dust, ozone, carbon dioxide, and so on) in the atmosphere, or in the Sun or the cosmic environment, will require techniques ranging from time-series analysis of the very long climatic records to mathematical modelling of the atmosphere and hydrosphere and many new types of observation (such as of oxygen and carbon isotopes). Some leading meteorologists in various countries today maintain that the problems of long-range weather forecasting on any time-scale will only be solved by mathematical modelling techniques, that there is no convincing evidence of any variability of the solar output (though the series of direct observations are somewhat short for such confidence) and that there is little point in attempting to reconstruct the past record of climate in any detail. At the other extreme, a conference held at Brown University, Providence, Rhode Island, in 1972 attended by geologists, palaeobotanists, marine biologists and others, including representatives of the oxygen isotope laboratories, two leading meteorologists and a physical oceanographer, took as its theme "The present interglacial, how and when will it end?" The verdict favoured by the majority⁵ was that "the natural end of our present warm epoch is undoubtedly near . . . Global cooling and related rapid changes of environment substantially exceeding the fluctuations . . . in historic times, must be expected within the next few millennia or even centuries . . .".

There is a manifest psychological difficulty in bringing together the type of mind represented by the different attitudes among scientists mentioned in the previous paragraph. But it must be done. And perhaps the most helpful of all avenues of research towards reaching a balanced understanding is to seek out the facts of the past climatic record. As T. H. Huxley wrote, one must "sit down before fact as a little child". The actual record of the past is, after all, vital to test the realism of the mathematical models.

The Climatic Research Unit at Norwich aims to map and publish the ascertainable facts of climate and circulation patterns prevailing year by year over the period of historical documentation, both before and after the introduction of meteorological instruments, and to work on meteorological analysis of the average conditions of more remote periods for which the data are derived from many kinds of fossil evidence. In this work, liaisons with the most diverse branches of science and learning must be cultivated. The CRU also reviews at regular intervals the current development of climate and experiments with methods and formulation of forecasts covering periods from some months to many years ahead.

It is known that several bodies in various countries engaged in activities involving planning for up to fifty years ahead have devised their own methods of arriving at the projections of future climate which they need and that some scientists have also produced forecasts for a similar length ahead. These forecasts have so far been based on one or other single line of evidence and have doubtless been regarded as less than satisfactory for this reason by their originators. Surprisingly, however, there is (as is probably quite widely known) a consensus among them of a trend towards a cold climate in the last decades of the present century.

There is an obvious need, and it is among the aims of the Climatic Research Unit at Norwich, to bring the various forecasting methods together, to review their physical assumptions and test their statistical basis with new data and so develop a wider understanding. It is hoped, in particular, to make progress by identifying quasi-periodicities in meteorological and

climatic data and relate them to the evolution of the global atmospheric circulation at the different phases of each. It should then be possible to recognize interrelationships which are not at present understood and to monitor each current cycle with an eye to anomalies and interference phenomena affecting it. To do this, it is necessary to set the long-term observational base of climatology on a firm footing. And it will be in pursuit of this that the Climatic Research Unit will make its first, and perhaps most distinctive, contribution.

A start has been made with building up an archive of historical observation data. Handsome gifts have been received from the remarkable wealth of early data from northern countries in the possession of the Danish Meteorological Institute. The help and advice of the Netherlands Meteorological Institute and of individual scientists in Germany, England, the United States and Japan, as well as of historians in France, Denmark and Norway, are also gratefully acknowledged. For the analysis of the climates in prehistoric times promising liaisons have been established with seven American universities, and with the sub-department of Quaternary research in the University of Cambridge as well as with the oxygen isotope laboratories in Cambridge and Copenhagen. An international meeting of research workers concerned with mapping the climatic patterns of the last ice age was held in the University of East Anglia in May 1973.

Compilation and meteorological analysis of the January and July maps of individual years from AD 1680, and compilation of seasonal data on maps back to AD 1200, begun when I was on the staff of the Meteorological Office, have proceeded far beyond the pilot stage, and the feasibility of the projects with these maps is established. The combination of linguistic, historical data sifting and meteorological talents required, and access to the great data banks in the Meteorological Office, Bracknell, and in the libraries in Copenhagen, London and elsewhere, is probably uniquely present in the staff of the CRU at Norwich, but the carrying through of the project is unhappily threatened by the present lack of financial means to continue employing the staff beyond 1975 or to provide the necessary ancillary posts.

Work at Norwich on the monitoring of the current development of the climate is at present limited to assembling data covering the northern and southern hemispheres that have already been collected and published by various meteorological services, to considering and analysing the data in various ways and to publishing a global digest based on this (CRUMB—Climatic Research Unit Monthly Data Bulletin). The unit is greatly indebted to the National Oceanic and Atmospheric Administration, the United States Air Force, and the Smithsonian Institution in Washington for gifts of daily weather maps of the northern hemisphere covering the present century and tabulated monthly climatic data for reference covering the past 100 years or more, and enjoys exchange arrangements with the Meteorological Office and the Deutscher Wetterdienst for analyses of the most recent observational material. Experimental forecasts are formulated several times a year and the results are to be assessed yearly. When the staffing position permits, it is hoped to stage a series of meetings to elaborate "forecasts" using exactly similar information and methods for years and seasons that are already past (and forgotten).

Once we have a secure base it will be possible to broaden our activities in monitoring current climate in many ways. There are plans for studies of the direct effects of certain types of pollution on climate, the effects of afforestation and of urban development, and the longer-term exchanges between sea and atmosphere over the northern seas.

¹ *Die Grosswetterlagen Europas* (Deutscher Wetterdienst, Offenbach/Main).

² Gedeonov, A. D., *Glav. geofiz. Obs.*, **211**, 68 (1967).

³ *Inadvertent Climate Modification (Report of the Study of Man's Impact on Climate)* (MIT, Cambridge, Mass, 1971).

⁴ *Man's Impact on the Climate* (edit. by Matthews, W. H., Kellogg, W. W., and Robinson, G. D.) (MIT, Cambridge, Mass, 1971).

⁵ Kukla, G. J., and Matthews, R. K., *Science*, **N.Y.**, **178**, 190 (1972).

Thermal Plumes in the Earth's Mantle

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Dr Tozer casts a critical eye over the concept of thermal plumes that is attracting a good deal of attention in the Earth sciences just now.

IN spite of declarations of belief by distinguished geophysicists at a recent Royal Astronomical Society meeting devoted to this subject that thermal plumes do not exist in the mantle¹, the matter seems to be of sufficient current scientific interest to merit a less theological treatment, and with this in mind I have been asked for my comments as an interested spectator.

I do not propose to waste more than the minimum of space on the question of the existence of thermal plumes, for the answer seems to depend on the way one chooses to use language. Besides, physical scientists are generally more interested in the usefulness of a concept rather than in any philosophical discussion of its reality or existence. When a geologist, at least the species that exists in the minds of physicists, if nowhere else, uses a technical term he seems to do so as a name for which the question of existence is a pure tautology. In particular there is no reason to think that the words "thermal plume" are now being used by them in any other than this sense to name collectively such diverse phenomena, inferences and ideas as, for example, that abnormally hot material rises from the bowels of the Earth to escape in the structures we call volcanoes; the process that produced the Hawaiian Islands; the pseudo-elastic structure of the deep interior of the Earth is not spherically symmetrical. As nobody has enumerated the criteria that have to be satisfied for inclusion under this particular name and, moreover, because in many minds such inclusion has come to be seen as an explanation of mysterious events, the number of "thermal plumes" shows the same tendency to increase with time as was shown in earlier epochs by "witches", "layers in the crust", "tectonic plates", and so on—my geological friends assure me that several tens if not hundreds of "thermal plumes" have now been discovered. One cannot deny on logical grounds this or any other attempt to classify geological phenomena, although on the grounds of previous usage, I think one can justifiably object to the nomenclature in this instance. I hope it is clear that a scheme to associate events or phenomena is no more a scientific explanation of their occurrence than is the fitting of a curve to a selection of experimental points, unless there is also a definite proposal as to the procedure by which it might be refuted.

Question of Definition

I suspect that the rather loose usage of the term "thermal plume" sprang from a desire to have an original theory that would seem to make more immediate contact with geological observations than thermal convection theory, and an unwarranted eagerness to dismiss thermal convection as the theory that only predicted velocity fields in the mantle that were as smooth and symmetrical as they were geologically unacceptable. But there is no doubt that in its original meaning the

term "thermal plume" did refer to convective flows—in a recent review by Turner² buoyant plumes and thermals are described as "a variety of phenomena related under the heading of turbulent buoyant convection from small sources" and in particular "a plume arises when buoyancy is supplied continuously". As a definition this description, with its tacit reference to plumes as flows of high Reynolds number, is too restrictive, I believe, to capture what one would like to express by the term "thermal plume" and at the same time too vague to decide whether dynamically similar flows might occur in the Earth's mantle. If one did take it as the definition, the fact that most authorities estimate the Reynolds number of mantle flows to be $\sim 10^{-20}$ could be taken as a clear judgment that thermal plumes are not a useful concept in this context. Perhaps a definition of thermal plumes which comes closer to expressing one's intuitive meaning is that it is a way in which one describes a thermal convective flow in which a velocity-dependent length, usually referred to as the thermal boundary layer thickness, is small compared with the linear dimensions of the system. This may be shown to be a convective flow for which the Péclet number $Pe = \nu L/K \gg 1$ where ν is a characteristic speed of flow, K the thermal diffusivity of the medium and L the linear dimensions of the system. Taking from geophysical observation $\nu = 10^{-7}$ cm s⁻¹ (3 cm yr⁻¹), $K = 10^{-2}$ cm² s⁻¹ and $L = 10^8$ cm, we get $Pe \sim 10^3$, which certainly satisfies our condition. One could still raise the objection, however, that this is only a necessary condition for the existence of thermal plumes and that the flow would not, for the following reasons, match the "thermal plumes" envisaged in the mantle (see, for example, ref. 3). In the first place, one notices that the Prandtl number, $Pr (= \nu/K)$ of mantle material is universally believed to be enormous ($\sim 10^{22}$) and, as $Pr^{\frac{1}{2}}$ gives the ratio of thicknesses of the viscous and thermal boundary layers, one sees that no large velocity gradients would be expected to exist at the limits of any attached or detached thermal boundary layer—a feature that has been taken to be characteristic of thermal plumes, and which is fairly clearly exhibited by high Péclet number flows in the atmosphere and ocean where the Prandtl number is 0.7 and ~ 7.0 respectively. Incidentally, the fact that a purely formal calculation of viscous boundary layer thickness in the mantle gives a length greatly exceeding the dimensions of the mantle would seem to belie some rather mystical talk that one part of the mantle is "decoupled" from another, but perhaps someone else could explain what is meant by that.

The second objection to thinking that the high Péclet number calculated here is sufficient reason for believing that the deep mantle has ascending plumes of hot material is based on an estimation of the thermal energy flowing into the mantle from the core. It will be recalled that one of the persistent difficulties of geomagnetic dynamo theory has been to find a source of energy that will sustain the necessary motions and at the same time be acceptable to geochemists. Translated into terms that are relevant to the present problem, one can call this the difficulty of supposing that less than $\sim 90\%$ of the heat flowing through the Earth's external surface has been generated throughout the mantle/crust region. Although there has recently been some change of attitude among geochemists about the possible

potassium content of the core, it is still hard to believe that the mantle/crust system has anything like as much heat entering at the bottom as is measurably leaving it at the external surface, and it is because of this difference in the spatial distributions of heat supplied and lost that one must expect the concept of a plume as a detached thermal boundary layer to be more descriptive of cold descending material near the external surface than of rising material. This asymmetry of the flow in comparable circumstances of volume heating and surface cooling has now been clearly demonstrated in model experiments⁴. Of course, the more one is prepared to confine convective motions, such as by rapid change of rheology with radial distance, to the outer parts of the mantle/crust system, the easier it is to justify a significant heating from below and a description of that motion in terms of both rising and falling streams containing thermal boundary layers. But the extremely large Prandtl number of the situation should warn one that the rising stream is no ordinary thermal plume, and it is obvious that such a more or less upper mantle flow cannot match all the attributes ascribed to "mantle plumes" by geologists.

From these remarks, it will be seen that a decision to use the words "thermal plume" to describe only those convective flows that are dynamically similar to certain observed flow phenomena that have been named thermal plumes in the atmosphere and ocean leads to the conclusion that they do not exist in the mantle/crust material. This may be the source of some misunderstanding. Although some geophysicists may quite justifiably feel that lack of dynamical similarity is sufficient reason to ban the words in a geotectonic context, they have not been clear enough in their own minds to point out that a mantle flow which locally has a reasonably close but purely kinematic similarity to atmospheric thermal plumes is still not denied by mantle convection theory. In fact, as I have spent some years in advocating that in the most plausible formulations of the mantle convection problem very narrow columns of rising and abnormally hot material can be expected as part of the general circulation, I should like to describe briefly this view of the convection problem because it may help to define our subject matter and establish contact with general geophysical theory.

Two Factors

In a study of the Earth's response, and indeed that of the other planets, to any geochemically plausible distribution of heat sources, one is compelled to include *ab initio* the variation of material rheology with temperature and the production of heat during any material deformation. These two factors are present, of course, in all the well studied laboratory systems, the atmosphere and oceans, but it transpires that they have no great significance for the understanding of those systems, save in certain very restricted circumstances where the lifting of a degeneracy that would otherwise exist is of prime observational interest. They can be added to a theory that initially ignores them as a kind of afterthought. In contrast, with planetary problems the numerical value of the constitutive function representing the material rheology is very strongly influenced by the flow itself, rather than just by the boundary conditions. This adds to the complication of making a precise theoretical analysis and destroys the possibility of close dynamical similarity with any other system differing much in size. Limited calculation has already shown that the response of a planet to its heat sources is quite different from what has been expected from certain oversimplified methods of analysis, for example, by studying it as a problem in heat conduction theory or as equivalent to heat transfer in a body with a particular temperature-independent rheology. The salient results of the efforts to elucidate the steady state response for a set of planetary models that would cover the wide range of possible planetary materials can perhaps be best summarized in the language of control theory. One finds that the heat transfer

process acts as a powerful negative feedback to keep the horizontally averaged numerical value of the rheology function below a certain depth (expressed purely dimensionally in units of viscosity) nearly independent of its particular dependence on temperature.

Further indication of the strength of this feedback is shown by calculations of the rapidity with which a postulated non-steady state situation relaxes to the steady state. This turns out to be typically of the order of a few hundred million years, though the non-linearity of the problem makes it not always possible to characterize relaxation from an arbitrary initial state quite this simply. The situation in the Earth is further complicated by the influence of hydrostatic pressure on the rheology, but calculation showed quite clearly that the average "viscosity" below a depth of a few tens of kilometres and above a few hundred kilometres would be tightly constrained to be about 10^{20} to 10^{21} poise (ref. 5). Although this particular figure and other predictions of the steady state solutions give a quantitative interpretation and coherence to a wide set of terrestrial observations, such a strong constraint on upper mantle rheology seems at first to make the existence of magma, that is, material with "viscosity" at least 10^{14} times less than the figure just mentioned and the *sine qua non* of geologists' "thermal plumes" (?), even more problematical than with the earlier theories of a planet's response to its heat sources.

Magma Formation

Ever since Lord Kelvin showed that the Earth as a whole has a higher rigidity at tidal frequencies than that of steel at room temperature, scientists who have tried to answer the problem of magma formation can be broadly grouped into two classes. In the first were those anxious to preserve a spherically symmetric view of the Earth's interior who were forced to assert that Earth material could exist in a state that simultaneously combined the requisite rigidity and viscosity, whereas the others saw magma generation purely as the result of some local anomaly in material composition. I believe a less *ad hoc* explanation of magmatism that makes it a part of the overall global response has now been found in an interplay of viscous dissipation of the internal motions and the decrease of "viscosity" with a rise of temperature. It may be shown that the ratio of the heat produced by the viscous dissipation of a motion to a "primary" heating that is assumed responsible for the existence of that motion increases with the size of a system and approaches 10^{-1} in a typical planetary situation. A value of $\sim 10^{-8}$ in any practical thermal convection experiment ensures that the interplay referred to never leads to any observable consequences there, but I believe it is possible to make some safe conjectures about the results of scaling upwards in size at fixed Rayleigh number, the empirical configuration that at the moment seems the closest simulation of a planetary situation—I refer to an internally heated plane layer with Rayleigh number $\sim 10^6$ and Prandtl number $\sim 10^2$ that is cooled from above and thermally insulated below. One can expect that because any plausible planetary "viscosity" function decreases in value with increasing temperature, the spatial inhomogeneities that are present in the rate of shear field on a laboratory scale become both more enhanced and concentrated as the depth of the layer is increased. As it is now known that on a laboratory scale the average rate of shear throughout descending material in internally heated flows at such Rayleigh numbers is much higher than elsewhere⁴, one expects the effect of viscous dissipation in reducing the "viscosity" and sharpening the peaks of the rate of shear distribution to be first manifest there, and that there would be some concomitant redistribution of the heat flow at the upper surface that relatively enhances its value over descending material. It is possible to show that for layers whose depth exceeds about 10 km and whose average viscosity is $\sim 10^{20}$ poise, the shearing within the descending material

would have evolved to a type of quasi-discontinuous slip across a zone that is extremely narrow compared with the depth and which contains material with a "viscosity" that is typical of magma (with all that that implies about a temperature anomaly). One senses that such zones might well grow out of relatively small inhomogeneities of a flow that was at least on the scale of the upper mantle, but the asymmetry of the situation that is due to volume heating and surface cooling leads to the prediction, probably the most characteristic of this theory, that magma production is principally associated with descending material.

It is unfortunate that the purely technical difficulties of handling a problem that involves such a wide range of length scales have precluded a more detailed development of this theory of magmatism, but among its most attractive features that seem to match observation and which should be sufficient bait for any intrepid theoretician are: (1) A natural explanation of the non-random distribution of volcanoes and their positional correlation with descending material. (2) A resolution of the rigidity-cum-viscosity paradox by the prediction that almost all upper mantle material is constrained by the heat transfer process to have a viscosity $\sim 10^{20}$ poise, and that the thermal/rheological condition of matter that we call magma can only obtain in extremely narrow zones "thermal plumes?". (3) The rate at which the total viscous dissipation can produce magma, that is, reduce the viscosity of mantle material from $\sim 10^{20}$ to $\sim 10^6$ poise, is a few cubic kilometres annually—a figure that has also been estimated from geological observation.

I would like to think that such figures for the total magma production rate would have a calming influence on the much publicized problem of the site of the Hawaiian Islands. It will be recalled that their location at the centre of what has come to be known as "a rigid plate" has caused much consternation, though I cannot help thinking that this problem is rather like that of a man who insists that the Earth is flat, and who is constantly perturbed by the disappearance of ships over his horizon. The volume of material that comprises the Hawaiian Islands is indisputably enormous to the human mind, but represents an exceedingly small fraction ($\sim 0.1\%$) of the magma that would have been produced in the period of their formation

and existence. I cannot think of any other problem involving finite deformation theory where failure to account for the disposition of such small fractions of a total mass flux is regarded as a theoretical failure or indeed of such scientific importance.

New or Old?

Having accepted the invitation to write an article that was supposed to comment on the theoretical respectability or otherwise of thermal plumes in the mantle, I have found it extremely difficult to decide whether theoretical geophysics was being asked to comment on a new phenomenon or on the current jargon for the very old and real problem of magma production. One has been constantly in the position of having to define what others mean by the title in order to make intelligible comment and I am very conscious that, in trying to do so, I may well have knocked down or elevated my own Aunt Sallys. My fears in this respect were not helped by a recent editorial in *Nature*⁶, which commented that some phenomena that had been attributed to "thermal plumes" might well prove explicable in more conventional geological terms. It would be most helpful if someone would explain in terms that are meaningful to geophysicists in what respects the conventional geological pictures of rising magma differ from "a thermal plume". I would wholeheartedly agree with P. J. S. that "something is clearly going on" and that it is highly desirable to test alternative explanations of the data⁶, but if that is to amount to more than a pious hope, the protagonists of "thermal plumes" have to stop using the words as a name for phenomena or states of mind—for example, "that which makes the situation around Iceland more complex"—and frame a definition that is amenable to test.

¹ *Nature*, **243**, 192 (1973).

² Turner, J. S., *Ann. Revs. Fluid Mech.*, **1**, 29 (1969).

³ Deffeyes, K. S., *Nature*, **240**, 539 (1972).

⁴ De La Cruz Reyna, S., *Geofis. Int.*, **10** (2), 49 (1970).

⁵ Tozer, D. C., *J. Geomag. Geoelec.*, *Kyoto*, **22**, 35 (1970).

⁶ *Nature*, **243**, 491 (1973).

Applicability of Plate Tectonics to Pre-Mesozoic Time

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Plate tectonic theory has provided a synthesis to account for the geological development of the Earth's crust during the past 10% of its history. Doubts are now being expressed, however, about the applicability of this theory to the origin of some zones of deformed Precambrian rocks.

THE plate tectonic model¹ is, in the first instance, an instantaneous description of present-day crustal behaviour. It is recognized that the crust and the top few tens of kilometres of the underlying mantle (together known as the lithosphere) behave as a small number of rigid plates in motion relative to each other and to the rotation axis of the Earth¹⁻⁴. Virtually all seismic, volcanic and tectonic activity is localized near the margins of these plates and is associated with differential motion between them. The margins are of three kinds. At extensional margins two adjacent plates are moving apart as new oceanic crust is created, in general accompanied by formation of an ocean ridge. At convergent or destructive margins

two adjacent plates are moving towards each other and motion is generally taken up by one plunging by subduction beneath the other. Only oceanic parts of plates seem to be subducted to substantial depths. When continental material is involved at the margin of the active plate it is generally deformed and retained near surface, and the process is orogeny rather than subduction. At translational margins two plates slide past each other without creation or destruction of crust.

Inspection of the young parts of the ocean floor suggests that this style of behaviour is not unprecedented. Along a boundary between two given plates the conservative segments form a set of parallel arcs of small circles centred on a uniquely defined axis, but extensional segments are arcs of coaxial great circles—the axes of the two sets being ideally identical. Relative motions at such margins can be described uniquely as a rotation vector about this axis; the poles of this axis are called tectonic rotation poles.

Former alignments of extensional plate margins are determinable from the orientation of linear belts of oceanic crust of a given age within plates; in practice these are chiefly recognized from linear magnetic anomalies which again are arcs of great circles. Past alignment of conservative plate margins is betrayed by the traces of extinct transform faults. In oceanic crust these are recognized by offsets of magnetic anomalies or as fracture zones; in continental crust they may be exposed as large transcurrent faults; again they form arcs of small circles. In principle the tectonic rotation poles and angles that describe the motion of all plates can be determined in relation to extensional and conservative plate margins only as far back in time as the oldest preserved ocean floor with each plate. It is this limitation, together with the fact that destructive plate margins are not amenable to this precise kind of analysis⁵, that in principle restricts complete plate tectonic solution for the Earth's crust to the time interval since the formation of the oldest ocean floor preserved in the existing ocean basins (about 200 million years).

The reality is less than ideal, even for this interval of time. Seafloor spreading data are far from complete. Tectonic rotation poles calculated by different methods at a given plate margin do not always agree and are in many cases imprecise. These shortcomings are partially compensated by two factors. (1) The aseismic continental margins mark the initial break that later developed into an oceanic ridge, and their best fit provides a good estimate of the initial relative positions of continents bordering the aseismic oceans. The rotations involved in making these fits can be described by a single Euler pole and rotation angle (after Euler's theorem which describes the geometry of displacement)⁶. The Euler parameters are the vector sum of the tectonic rotations which, if known, would define the actual paths of relative motion in detail. (2) From the additive property of related Euler or tectonic rotations, motion between two plates separated by a destructive plate margin can be determined indirectly provided a route can be plotted between them which crosses only extensional and/or conservative margins. These analyses have *inter alia* enabled the relative positions of all continents to be plotted with reasonable though variable degrees of precision back to 200 m.y. and led to a reassembly of the principal continental fragments into a single continental mass Pangaea at that time (late Triassic). Because there are no indications of active plate margins of Triassic age within this reassembly, the analysis effectively takes one back to the beginning of the Mesozoic.

Palaeozoic

For pre-Mesozoic time there are no data from the oceans of the kinds available subsequently, which is why "seafloor spreading" is excluded from the title of this article. Current discussion of pre-Mesozoic continental drift is strongly reminiscent of the classical pre-1960s controversy about more recent drift, hinging on the interpretation of land-based

geological and palaeogeophysical observations. That classic controversy related principally to the geometrical fit of opposed continental margins, distribution of fossil floras and faunas, geographic continuity or discontinuity of crustal structures and other trends, and palaeomagnetism. Together they were strongly suggestive of continental drift, but fell short of being utterly decisive. Their partial failure, I suggest, derived from their undue reliance on facets of uniformitarianism which were, and remain, unproven—namely the permanence of modern-style climatic zonation to control organism distributions, and the permanence of the geocentric axial dipole character of the ancient geomagnetic field.

The new controversy is being conducted on similar lines but without the assistance of geometrical fitting. There are advantages of framing the problem in terms of the plate tectonic model even though that model cannot be tested as fully as it can for more recent time. First, the climatic zonation model and geocentric axial dipole field model are now more securely based as a consequence of their compatibility with plate tectonic interpretations for the past 200 m.y. Second, plate margins are recognizable geologically by distinctive features for which one can search each interval of the older geological record, and the plates existing during each interval are characterized by lack of contemporary internal deformation. (Much controversy, however, now hinges on whether these "distinctive" features are truly diagnostic as is often claimed, and as is discussed later in this article.)

Extensional plate margins, where they develop into ocean ridges, naturally leave little mark on the continents. Nevertheless their former presence may be suspected where pre-existing geological features are abruptly truncated (as, for example, on the west coast of Africa where orogenic belts and sedimentary basins are cut off at the continental margin, their former continuations now being found in eastern South America). Extensive flood basalts dating from the time of supposed rifting may be indicative, although they are not diagnostic. Nascent extensional margins (of which the East African rift system could be a modern example⁷) may be recognizable within continents if their development was stifled before reaching the ocean ridge stage; they are then not indicative of large scale relative motion but are of interest as signs of the operative process.

Conservative plate margins, transform faults, are suspected of being preserved as transcurrent faults on continents, but they seem to be rare. Destructive plate margins are expected on the plate tectonic model to be preserved within continents, even if they initially developed in ocean basins^{8,9}. It is orogeny and related continental magmatism which is believed to be diagnostic, rather than the mere formation of thick geosynclinal sequences of sediments¹⁰. If eugeosynclinal belts form at plate margins, then orogeny may follow immediately after deposition. But miogeosynclinal (or miogeoclinal) sedimentary wedges may form within plates at an interface between a continent and an ocean and will not be deformed at a plate margin unless the spreading regime changes: in that case there may be a substantial time lag between deposition and major deformation.

Rocks of the ophiolite suite are now widely regarded as former ocean floor and their occurrence in orogenic belts is strong evidence of plate convergence. But the severity of orogeny is such that little or no relics of ocean floor may outcrop, so that absence of ophiolites does not of itself deny convergence. Occurrence of island-arc type volcanism may also be diagnostic; whether the same can be said of flysch or molasse facies sediments is less certain and it may be that these are not even diagnostic of continental margins, let alone plate boundaries. Distribution of organisms may assist Palaeozoic continental drift interpretations where provinciality can be established, or where progressions of successively deeper water continental-edge faunal communities can be recognized¹¹.

The palaeomagnetic consequence of plate convergence is

confluence of the polar paths for the individual plates, and their continuance as a common path after convergence has been completed. This is analogous to the confluence of Phanerozoic polar paths for each continent towards the present poles on the present-day map, and is the converse of the divergence of late Mesozoic and younger polar paths on a map of Triassic Pangaea (Fig. 1).



Fig. 1 Stereographic map of the continents as they may have been situated at the beginning of the Mesozoic. Palaeomagnetic polar paths are shown schematically. Distinct early and middle Palaeozoic paths from Siberia, Europe-North America and Gondwanaland converge to a single late Palaeozoic/early Mesozoic track during which time this Pangaea reassembly may be correct. The divergence of the tracks from later Mesozoic time reflects the dispersal of continents toward their present positions. Slightly modified from ref. 22.

There are thus three independent suites of evidence which can be brought to bear on the subject of pre-Mesozoic continental drift—palaeontology/palaeobotany¹², “plate tectonic geology”¹³, and palaeomagnetism. To conduct the investigation in this fashion does not imply *a priori* acceptance of the plate tectonic model for pre-Mesozoic time, though it would be surprising if a style of behaviour which seems to have been universal for the past 200 m.y. were completely inoperative at any time previously. This has not always been agreed¹⁴, however, and as recently as 1970 Dietz and Holden¹⁵ seem to have regarded “Wegenerian” drift as a unique event in Earth history. On the other hand, 200 m.y. is less than 5% of the age of the Earth and the geological record within the continents stretches back to 3,000 m.y. (exceptionally to 3,400 or even 3,800 m.y.) and it would be equally indefensible to accept without question a ten or twenty-fold extrapolation of the model through time. The desirability of determining the overall style of crustal behaviour for the duration of the entire geological record is self evident; whether or not the plate tectonic regime operated throughout in its latter day fashion may emerge from investigation.

Palaeomagnetic and plate tectonic interpretations concur in the view that in the late Palaeozoic/early Mesozoic the conti-

nental crust was congregated principally in the single Pangaea group (Fig. 1). There may, however, have been exceptions. The most notable uncertainty relates to China, the age of whose juncture with Siberia is not very well established, but other peripheral slices of Asia, Australasia and elsewhere may also have been separate.

That the Pangaea situation was ephemeral has been argued on general grounds by Holmes¹⁶ and on specific geological^{17,18} and palaeomagnetic evidence¹⁹, all of which visualizes at least three continental groups in the earlier Palaeozoic²⁰⁻²³ (Fig. 1). That Siberia was one of these, having distinct geological and palaeomagnetic records before being sutured to Europe at the end of the Palaeozoic, has been argued by Hamilton²⁴. The Caledonian and northern Appalachian orogenic belts have been identified as the site of a “Proto-Atlantic ocean” between Europe and North America, with Greenland and the extreme north-western margins of the British Isles attached to the latter^{17,25} but the palaeomagnetic evidence for this is not conclusive²⁶. The size and timing of the evolution and eventual disappearance of this Caledonian ocean remain uncertain. Structural and volcanological arguments have been cited to show that it was wide in Ordovician time^{25,27} and that oceanic crust still persisted in parts in the early Devonian; other chiefly petrological²⁸ and palaeomagnetic evidence, however, seems to indicate that it was already narrow by the mid-Ordovician and at the time of writing it remains plausible that Europe and North America were fairly close throughout the Palaeozoic. That is why I have referred to a few “continental groups” rather than attempting to specify the number of early Palaeozoic continents. The third of the three groups (after Siberia and Europe/North America and not knowing the whereabouts of China) seems to have been comprised of the Precambrian and Palaeozoic heartlands of South America, Africa, Antarctica, India and Australia assembled together and being cut by no orogenic belts younger than 500 ± 50 m.y. This is essentially the Gondwanaland²⁹ recognized in terms of continental drift by du Toit³⁰ for late Palaeozoic and Mesozoic time. The convergent plate margin(s) subsequently suturing it to North America and/or southern Europe probably lay within the Hercynian-Alleghenian orogenic belt, and various lines of suture within it have been suggested³¹, but precise demarcation must await a better understanding of the Palaeozoic geological setting of southern Europe.

Rates of polar shift determined from palaeomagnetism cannot be transposed into rates of crustal drift, without making assumptions about the relative positions of the plate and the Euler and palaeomagnetic poles. There is rarely any evidence for these relationships although some limits may be definable if well defined segments of polar paths are found to form arcs of great or small circles. It is possible that “quasi-static intervals”³², when the palaeomagnetic pole seems to have been stationary, may be due to proximity of the Euler and palaeomagnetic poles to each other, rather than to cessation of plate motion. Nevertheless the timing of polar shift can be equated with the time of maximum plate motion and it is interesting to note that in the examples so far investigated (Caledonides and Hercynides) this seems to predate the climax of orogeny by some tens of million years. Hence it may be correct to distinguish an earlier phase of major plate motion and island-arc type orogeny, from a later phase (continental collision type orogeny) in which relative motion of the converging plates is relatively small. The former is the more obvious palaeomagnetically and the latter is the more easily recognized geologically. The apparent long duration of the later phase seems to indicate that continental collision does not put an immediate stop to convergent motion in the underlying asthenosphere.

Thus hazy pictures are beginning to emerge of continent distribution back to 500 m.y. or so. But that is still only seven or eight per cent of the whole geological record; there is still a long way to go, and progress becomes more difficult as the record becomes more and more fragmentary.

Precambrian

Such evidence as there is for earlier time is principally confined to a few large regions of Precambrian crust which have been especially well studied. Moreover the number of independent lines of evidence is reduced in default of widespread faunal occurrence. The geological map abounds with older orogenic belts, each of which may be suspected as a convergent plate margin, and intervening stable cratons which remained undeformed during long periods when they may be regarded as plates in their own right.

Burke and Dewey³³ have cited occurrences of tholeiitic and spilitic pillow lavas, cherts, andesitic and dacitic volcanics and ultrabasic rocks as evidence of destructive plate margin activity and large scale subduction of ocean floor during continental collision along various segments of the Pan-African and some older orogenic belts in Africa, and have inferred that this analogy can be extended elsewhere.

Geochemical evidence of characteristic island arc-type processes is persuasive³³ and on this view reactivation of continental crust in these zones is ascribed to thickening of continental crust following collision, while the extraordinarily broad metamorphic belts adjacent to these zones are likened to the lower half of the thick Himalayan crust at the present time. Although these arguments are at their weakest in defining the amount of convergence involved, Hurley³⁴ has rightly remarked that application of the modern plate tectonic analogy to the exceptionally long-lived orogenesis of many of the older belts requires enormous amounts of motion and the destruction of hemisphere-sized oceans.

The contention of Holmes³⁵, Kennedy³⁶ and many others³⁷⁻³⁹ that the Pan-African and older orogenic belts in Africa were essentially intracontinental stemmed principally from recognition that in some cases pre-existing geological features were not substantially offset by them, and that in others progressive correlation of facies and deformation phases was possible across entire belts. (Interestingly most of these authors refer to "mobile belts" rather than "orogenic belts".) On this model reactivation of older continental crust at orogeny is ascribed to exceptionally high subcrustal temperatures and the crust need not have been of abnormal thickness. The finding that the supracrustal rocks are underlain by continental basement is not of itself convincing evidence of purely "intraplate" orogeny, although the preservation of relict areas of granulitic basement tends to support this view. Moreover, preservation of relict oceanic crust is by no means assured in plate margin orogeny. Realignment of pre-orogenic geology could be achieved if orogeny were always the culmination of a seafloor spreading/subduction cycle at the end of which the margins resumed their pre-spreading position¹³. Some such reversibility in the spreading process seems to be implicit, for example, in the Proto-Atlantic ocean models^{17,25}, but there is no hint that controlling forces of the required kind have ever existed; indeed the finding in young ocean basins that tectonic rotation poles move quite substantially makes it inconceivable that the spreading path could be accurately retraced in any future convergent phase.

Palaeomagnetism offers the prospect of resolving this evident impasse because, as Piper *et al.*⁴⁰ have pointed out, the various tectonic hypotheses predict utterly distinct patterns of palaeomagnetic observations. Palaeomagnetic field directions inferred from a single plate should be consistent with a single apparent polar wander path (polar path) relative to that plate. Polar paths from plates in unrelated motion should differ drastically. If a set of plates were loosely aggregated, predictable similarities and differences in their polar paths should be evident.

Late Precambrian polar paths (for 1,000 m.y. onwards) from Africa and the Canadian Shield are utterly different, as would be expected if they were separate plates⁴¹⁻⁴³, and it may be that they had always been separate^{41,42}. Although mere similarity in size and shape of two contemporaneous polar paths does not prove that the two should be superimposed

throughout their length, Piper⁴³ has demonstrated that if this is done for the time interval 1,400 to 1,000 m.y. it follows that the Canadian and African shields were adjacent during that time, and could have been parts of the same plate. This is not to imply that plate tectonics was inoperative between 1,400 and 1,000 m.y.; such a conclusion would only be tenable if palaeomagnetic and geological evidence from *all* surviving crust of that age were compatible with aggregation in a single plate. Indeed it is most improbable that that will prove to be the case, because it already seems clear that the Grenville province was converging towards the rest of the Canadian Shield during this same period of time⁴⁴.

Alongside this evidence of relative plate motion at least 1,300 m.y. ago, the speculation by Piper⁴⁵ that several of the cratons of Africa were already in essentially their present relative positions by 2,200 m.y. is now supported by a wealth of new data⁴⁰. A simple part of this evidence is illustrated in Fig. 2, in which palaeomagnetic poles derived from the Kaapvaal, Congo and West African cratons seem to fall on a common path. Inevitably there are statistical uncertainties associated with each estimated pole, and the time coverage from individual cratons is incomplete. Hence the polar path in Fig. 2 is drawn as a swathe whose width gives some idea of the amount of relative motion between cratons that the data will tolerate (say, 1,500 km). It shows that the destruction of a Mediterranean-sized ocean is tolerable, but not one as large as the modern Atlantic, and it flatly eliminates the possibility that hemisphere-sized oceans have been destroyed between these cratons. The same conclusions would be drawn if one looked at data for any interval of a few hundred million years. Taken overall, the data for 2,200 m.y. to the present all conform to such a long and complex common polar path that even 1,500 km is likely to be a gross exaggeration of the amount of distortion. The implication is that Africa has been a single continent since at least 2,200 m.y. because even the possibility of "reversible" plate motions (opening and then closing an ocean so that the margins resume their original fit) is eliminated unless the reversibility was immaculate to a degree which is hard to credit.

It may be contended that the palaeomagnetic data and the evidence of structural continuity are still inadequate to prove

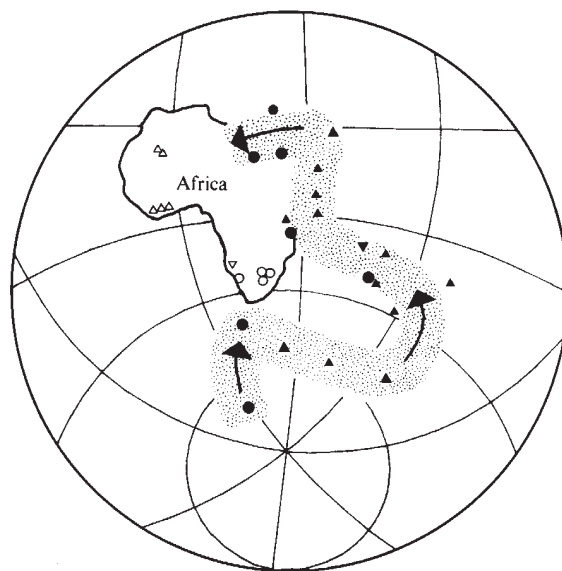


Fig. 2 Stereographic map of palaeomagnetic sampling areas (open symbols) and pole positions (solid symbols) from African rocks in the approximate age range 2,300 to 1,950 m.y. Kaapvaal craton, circles; Congo craton, inverted triangle; West African craton, upright triangles. The small symbols imply that poles were calculated from measurements at single localities only; large symbols denote the mean from several localities. Modified from ref. 40.

that the orogenic belts under discussion formed entirely *in situ* within a pre-existing continent. But the case is sufficiently strong to justify questioning the extreme plate tectonic view that the occurrence of orogeny necessarily implies that large areas of ocean floor have previously been subducted on its site, and in the remainder of this article a number of alternative origins and mechanisms will be entertained. I would emphasize, however, that the converse proposition that destructive plate margins are only preserved in orogenic belts is not being queried here and also that it is merely a matter of taste whether one regards deformation within a continent as consistent with identifying that continent as a "plate". I am, therefore, not deeply concerned whether the following propositions are regarded as alternatives, or supplements, or merely aspects of plate tectonics as presently conceived.

First, it could be suggested that the old mobile belts do have modern equivalents, but that none are exposed, being in the lower crust. The distinction between Precambrian cratons and mobile belts would then be merely in level of exposure.

Second, internal deformation of continental crust might have been more common in the Precambrian than since. This proposition has a number of variants. Perhaps Precambrian continental lithosphere was weaker because it was thinner. The sharper gradients of temperature-sensitive metamorphic zones as found in Archaean rocks would support this view, but it is doubtful whether this state of affairs persisted as late as 550 m.y. and in any case it is doubtful whether the lithosphere could have been thin enough to buckle under the forces operating on modern plates.

Perhaps it was weaker because it was hotter. The occurrence of extensive volcanicity and plutonic magmatism within present crustal plates is currently being ascribed to localized thermal highs^{46,47}. Whether these are to be identified with plumes of exceptionally hot mantle material is not critical in the present context. What is noteworthy is that the proponents of Precambrian intracontinental orogeny, metamorphism and magmatism have invoked a similar cause. Adopting for a moment the mantle plume picture, it is being suggested that the plumes migrate linearly with respect to the overlying lithosphere and hence might be capable of producing linear belts. The precision of dating radiometric events within these belts has not been sufficiently precise to detect the lengthwise age variation which is implied in this model, but this is certainly within the capability of current methods. Although this model may be capable of producing the actual metamorphism and magmatism it remains to be seen whether it can be reconciled with the compressive crustal pattern required to produce the regional orogenic deformation.

Perhaps weaker parts of continental lithosphere deformed as a repercussion of continental collision elsewhere at a plate margin, however distant. Such repercussions might take a long time to dissipate themselves, and the braking effects normally supposed to operate after continental collision at a plate margin may not apply.

Perhaps convergent motion in the asthenosphere could be maintained for long periods, or even initiated, beneath continental lithosphere, despite the absence of modern examples. After all, it is implicit in plate theory that divergent asthenosphere motions can be established beneath continental lithosphere and, until rupture actually occurs, must be decoupled from it. Likewise after the initial contact of two colliding continental margins, convergent motion in the underlying asthenosphere must be at least partially decoupled unless subsequent phases of orogeny exclusively take up the inertia of the system.

All these alternatives, and probably others, would be compatible with the long deformation histories of some Precambrian mobile belts, and, most important, for their observed superposition and cross-cutting relationships. Given small amounts of relative motion they might also be compatible with the limited occurrence of volcanic and ultrabasic rocks otherwise associated with convergent plate margins.

In conclusion it is worth repeating that the palaeomagnetic evidence which largely stimulated the latter part of this discussion does not contradict the hypothesis of plate behaviour of the crust. On the contrary it strongly suggests that large areas of crust did behave as coherent units—perhaps rather too flexible to be called plates, but sufficiently rigid to maintain their shape and entity. Clearly the investigation of pre-Mesozoic global tectonics is destined to pose problems for many years yet. It seems at present that (contrary to experience for more recent geological times) palaeomagnetism will provide the more refined information, coming as it will chiefly from undeformed plate interiors. The geological evidence, by contrast, is likely to be derived principally from deformed zones and for that very reason will be the more difficult to interpret. The older palaeomagnetic data are, however, not expected to be superior to the younger, so that a picture of the behaviour of the Precambrian Earth as precise as that now evident for the Mesozoic and Cainozoic is not yet in prospect.

African palaeomagnetic and structural investigations in the Department of Earth Sciences, Leeds University, are supported by the Natural Environment Research Council. I thank colleagues in this department for discussions, particularly R. A. Cliff, M. P. Coward, K. Lomax, J. D. A. Piper and R. M. Shackleton. F. J. Vine made helpful criticisms of an early draft of this article.

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The Tempo of Human Evolution

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Dr Bishop here discusses the isotopic dating methods used in developing a time-scale for continental deposits yielding hominid and other mammalian fossils from the past 5 million years.

IN the first programme of the BBC television series *The Ascent of Man*, the recovery of numerous hominid fossils from East Africa since 1959 was described by Dr J. Bronowski as being "as exciting as the discoveries in physics before 1940 and those in biology since 1950"¹. Many of these finds were announced in *Nature* and have received wide coverage elsewhere. Important as the discoveries certainly are, neither taxonomic arguments over that which we call *Homo* nor discussion concerning sophisticated reconstructions of palaeoenvironmental settings should obscure progress in a third field.

This is the investigation of man's speed of "ascent". It calls for knowledge of rates of change in morphological characters of bone and tooth seen in the comparatively few specimens which successfully ran the gauntlet of scavenging by predators and breakage by weathering processes to become buried and fossilized. Even fewer survive to be collected, or excavated, and take their place among that select band of fossils situated somewhere along the line which terminates in modern man. The subject involves the establishment of a time-scale against which to view both the fossils and cultural evolution, as inferred from stratified lithic or other remnants of the litter so unthinkingly but usefully contributed by prehistoric men as indicators of the "tempo of cultural change"².

This review is written by a geologist "consumer" of isotopic dates rather than a specialist geochronologist. In it I try to assess the progress and limitations of radiometric dating methods as applied to the past five million years (Pliocene plus Pleistocene). This is the time-span into which most of the new finds of hominid fossils and proven artefacts fall. An understated but important feature of the recent East African discoveries is that they occur in mapped sequences of superimposed strata. This permits fossils and artefacts to be placed immediately into a relative age sequence. Subsequently dates, and dating methods, can be checked against each other and subjected to the simple but effective judgment of satisfying the law of superposition. Individual dated sequences for rich artefact- and hominid-bearing strata principally from the Omo Valley (Ethiopia), the Rudolf and Baringo Basins (Kenya) and Olduvai Gorge (Tanzania) combine to provide a time-base against which to view human evolution together with rates of change in other critical biological and physical parameters.

General Problems

Several problems beset those who would construct such time-scales, quite apart from technical difficulties associated with specific methods.

(i) Continental and marine time scales. Hominid fossils and artefacts are almost invariably preserved in continental environments. Thus they have only a patchy distribution in space and time. A reasonably complete record, and an inferred time-continuum, may be represented in carefully selected marine sequences and in the igneous substrate of persistently spreading ocean floors. It is difficult to extrapolate dates from sea to land, however. Conversely, most of the firmly dated horizons for use as time-lines in calibration of strata yielding fossil vertebrates have been established for volcanic rocks in well exposed, but incomplete, continental sequences. Fortunately, palaeomagnetic stratigraphy, as described in more detail later, is proving a useful time-straddle between continental and marine sequences.

(ii) Dating pedigrees. The various unsuccessful attempts to date fossil bone have been reviewed recently³. A reliable dating technique has yet to be established for fossil bones older than about 30,000 yr BP. Radiocarbon dating has been applied with apparent consistency only to the collagen fraction of bones younger than 30,000 yr BP³. Such dates would be given a pedigree of A1 under the system of grading suggested by Oakley⁴. Some authors^{5,6} have cautioned that fossil bones, like molluscs, are open systems and hence likely to give unreliable ages. Although ages may seem to be valid they should not be accepted without careful scrutiny including stable isotope measurements and investigation of geochemical aspects of their sedimentary environments. For bones older than 30,000 yr, an A1 pedigree, implying a direct determination on the specimen itself, is not yet possible. For artefacts, the date at which they were made can be determined for specimens that have been accidentally heated in hearths, or by thermoluminescence dating of recent specimens. Normally, however, a direct date on the specimen gives only the age of formation of minerals in the rock (or date of death of the wood) from which it was fashioned.

Such considerations mean that most dates relating to hominid or artefact specimens occur in the lower grades (A2 to A4) on Oakley's scale⁵. A statement outlining the stratigraphic relationship between the material to be dated and the fossil whose age is required should be recorded in the pedigree.

Although the dating grades are largely self evident they are so frequently ignored that they deserve brief repetition. Many dates fall into an A2 category and are based on the age of some source deposit in which the fossil was found (such as potassium-argon ages on crystals from a primary ashfall stratifying contemporary hominids). A3 ages

inferred by stratigraphic correlation from one locality yielding specimens to another where isotopic dates have been obtained are quoted frequently, however, without a qualifying pedigree.

Finally, A4 grade ages, inferred by reference to some theoretical consideration, are commonly used but can be dangerous unless carefully qualified in the pedigree. Palaeomagnetic reversals of polarity may be placed into this category but are proving useful where based on evidence from long successions for which detailed palaeomagnetic polarity studies, ideally with some isotopic dating control, have been carried out. World-wide climatic changes recorded as stratigraphically recognizable events that are inferred to have time-significance may also be used (such as variations in palaeotemperature established by studies of foraminifera in deep sea cores or changes in tree pollen spectra of continental interglacial deposits). But such changes have the disadvantage that they are frequently time-transgressive.

(iii) Climatic confusion. With care climatic data can be used in extrapolation to provide, at best, ages of grade A4 pedigrees. It cannot be emphasized too strongly that inferred climatic cycles require calibration by isotopic dates to check firmly established stratigraphic data against such concepts as the Milankovitch hypothesis or similar theories of world-wide climatic change. Climatic theory is not in itself a satisfactory basis for setting up a time-scale although it may assist in stratigraphic correlation. Many workers hope that the Milankovitch or some similar hypothesis, based on correct prediction of periodicity of climatic change from knowledge of astronomical variables, can be developed into a dating method. This may eventually prove to be the case but at present it is premature to use such an approach as the basis for a time-scale, as attempted recently by Evans⁷.

Review of Methods

For more detailed information the reader should consult textbooks or reviews of dating methods based on quantification of the rate of decay of radioactive isotopes of carbon, potassium and uranium (radiometric "clocks")^{4,8-10}. The brief outline and selected references given here for each method concentrate on recent advances and their relevance or limitation in relation to dating continental deposits through the past 5 m.y.

(a) Natural radiocarbon. This method was put on a practical basis as long ago as 1949, following several years of research, and after recognition of its potential by Libby¹¹. Results are collated regularly in the journal *Radiocarbon* and revised details of techniques and problems are published from time to time¹². The range of the method is from the present to about 25,000 yr BP in general, but to 50,000 yr or more in ideal cases, for materials that have been in equilibrium with the carbon dioxide of the atmosphere. Following refinement of the half-life of ¹⁴C, recent research has concentrated on testing basic assumptions: (1) that the rate of formation of ¹⁴C is constant, (2) that ¹⁴C is well admixed throughout the reservoir of atmosphere and biosphere, (3) that further ¹⁴C is not added to the "closed" system after death of the organism which is the source of the dating sample. The range of local variations in (2) can be monitored at the present day. Variations of (1) with respect to time are of particular relevance to archaeologists¹³, for, as shown by Stuiver and Suess¹⁴, they upset the usually assumed relationship that one ¹⁴C year is the equivalent of one year on the prehistoric calendar. Correction curves, based on dendrochronology, comparing ¹⁴C dates for the range 1,500 to 4,500 BC with "actual" age in tree ring years were published by Suess^{15,16} and have been discussed by others¹⁷. Recently Ottaway and Ottaway¹⁸

have applied corrections from the Suess curve to various Neolithic cultures and draw attention to the need to take into account evidence from carefully excavated, well stratified sequences in addition to tree ring evidence in looking objectively at past changes in the concentration of atmospheric ¹⁴C.

Several workers^{5,6} have stressed recently the need to minimize errors in dating young samples by establishing initial ¹⁴C/¹²C ratios. But most errors in the application of radiocarbon dates to the stratigraphic record have arisen because of lack of recognition that the system to be dated is not closed⁵, or from failure to collect specimens carefully in order to avoid contamination, particularly by young carbon in old samples. For example, "modern" molluscs from Lake Edward in Uganda yielded ages of about 3,000 yr owing to the presence of old carbonate from calcareous volcanic ash after archaeological "dating" had suggested that radiocarbon ages for the Lake Edward Basin were too old. Conversely, Page¹⁹ recently published apparent ages well within the maximum range of radiocarbon dating for several peaty interglacial deposits in Britain. Despite a pronounced clash with stratigraphic, geomorphological and archaeological evidence he ascribed a young age to the Hoxnian, its contained fauna and artefacts, and to other interglacials. The most probable explanation, suggested by several lines of correlation with older dated sequences and supported by "infinite" ¹⁴C dates on other specimens from the deposits sampled by Page²⁰, is contamination of the samples yielding the anomalously young ages by young carbon.

These two examples emphasize the fact that not all materials have the same degree of reliability as potential dating samples. In order of increasing difficulty of determining the accuracy of the age, the best materials for dating are: wood; grain, cloth, hides and so on; charcoal; bone collagen. Peat, dispersed organic matter and various forms of carbonate occur towards the end of the list.

Radiocarbon dating has contributed greatly towards a schedule of events through the past 50,000 yr (refs 21-23, for example). A different scale of accuracy applies when radiocarbon dates calibrate only the upper portions of cores and sequences. A standard rate of operation of some process (usually deposition of sediment) is then used to extrapolate a time-scale without fixed points to periods before the past 50,000 yr. This, however, applies principally to deep sea cores and to attempts to calibrate climatic change for periods spanning the early part of the last glaciation and the last interglacial. By contrast, the histogram constructed by Isaac² draws attention to the fact that in continental deposits containing artefacts or hominid fossils there is a sharp "cutoff" of reliable isotopic dates at the threshold of radiocarbon dating. Before this there is a hiatus of over one million years before potassium-argon dates become comparatively abundant and occur in long stratigraphic sequences.

(b) Potassium-argon. The decay of the radioactive isotope of potassium (⁴⁰K) with production of radiogenic argon (⁴⁰Ar) is widely used for dating rocks back to the age of the Earth. During the past ten years it has been applied successfully to younger rocks in age ranges up to 1 m.y. and less. One of the early applications to Quaternary strata was by the Berkeley Laboratory, University of California, in establishing an age for the skull of *Australopithecus (Zinjanthropus) boisei* from Olduvai Gorge^{24,25}. The chief technical problems are in measuring the relatively small amounts of radiogenic argon that accumulate in young samples and in determining the fraction of ⁴⁰Ar that is of atmospheric origin. In spite of these difficulties in dating young rocks the method potentially overlaps the older range of radiocarbon dating. In practice, however, too few well substantiated dates fall into this time-span for the two methods to be checked against each other.

Details are discussed in refs 26 and 27, and problems of contamination in ref. 28. Recent developments of $^{40}\text{Ar}/^{39}\text{Ar}$ single sample dating and step-heating methods^{29,30} are yielding more refined dates for the late Tertiary and Quaternary. The $^{40}\text{Ar}/^{39}\text{Ar}$ approach offers a way of overcoming inaccuracies caused by sample inhomogeneity as it measures potassium content for the same sample on which the ^{40}Ar determination is eventually made. This is in contrast to the conventional method.

Fitch³¹ has reviewed the problems of assessing geological error and of selecting suitable dating samples. Most likely sources of error again involve sampling of potassium-bearing minerals which are incorrectly assumed to be closed systems retaining all the radiogenic argon formed since the mineral crystallized. Loss of some of the gas may arise through chemical weathering, physical strain or post-formational reheating of the rock. All result in discrepantly young ages. Inherited "excess" argon can cause discrepantly old ages but plotting of $^{40}\text{Ar}/^{36}\text{Ar}$ against $^{39}\text{Ar}/^{36}\text{Ar}$ isochrons offers a means of establishing the presence of excess ^{40}Ar and of possibly eliminating this problem²⁹.

Localities yielding potassium-rich rocks, or crystals, suitable for dating in clear and close relationships to artefacts and hominid fossils are limited. In this respect the volcanics of the East African rift system and areas flanking it are particularly favoured. Olduvai Gorge Bed I with 35 potassium-argon dates yielding a mean age of 1.82 ± 0.13 m.y. is the most accurately dated hominid-bearing unit beyond the range of ^{14}C dating³². Some 100 feet of strata comprising Bed I and Lower Bed II in the eastern part of the Gorge were probably deposited in 130,000 yr or less³². But no trend from older to younger is discernible in the dates for potassic minerals from tuffs through this thickness of strata. Although a sequence of fossils and artefacts can be derived from the known stratigraphic position of the finds³³ no rates of change can be established within the sequence because of lack of resolution of individual dates. This illustrates the need for caution to avoid placing undue weight on single or spasmodic potassium-argon dates.

Tuffs in the hominid-bearing Shungura Formation of the Omo Valley in southern Ethiopia have yielded a series of six dates for different horizons from 3.75 m.y. to 1.84 m.y., all in correct order of superposition³⁴. A similar set of inter-related dates for the succession in the Baringo area of the Kenya rift, with its younger members yielding artefacts and hominids, can be checked against a long stratigraphic sequence, thus revealing any "misfit" ages³⁵. Fitch³¹ has concluded that "successful potassium-argon dating requires scientific team-work and that isolated dates unrelated to a known regional chronology are unreliable". As more hominids and artefacts are excavated and the dating of these sequences is refined, rates of change should be discerned.

(c) Geomagnetic reversals. Although not in themselves a dating technique, changes in polarity of the Earth's magnetic field as recorded in both igneous and sedimentary rocks have proved extremely useful as a vehicle for extrapolating potassium-argon dates from well-dated into largely undated sequences.

The development over the past ten years of the investigation of changes in the polarity of the Earth's magnetic field and their calibration by potassium-argon dating has been summarized by Dalrymple³⁶. This has resulted in a detailed geomagnetic reversal time-scale for the past 4.5 m.y. This period is subdivided into phases of normal and reversed magnetic polarity of which the major ones are termed epochs and shorter ones events³⁷. Rocks formed during times of reversal of the direction of the field, at epoch and event boundaries, have been located in both igneous and sedimentary rocks in marine and continental environments. The resulting geomagnetic "time-scale" has been calibrated by almost two hundred $^{40}\text{K}/^{40}\text{Ar}$ dates which meet stringent requirements as to their accuracy.

The method has important applications in providing a time-scale for continuous sequences in deep sea cores and in assessing comparative "rates" of seafloor spreading. It also has the advantage that "refined" dates for reversal boundaries can be applied to incomplete continental successions providing that the typical "fingerprint" of a portion of the geomagnetic reversal scale can be recognized, preferably with confirmatory potassium-argon dating to help identify with certainty a particular reversal boundary or boundaries. For instance, this has proved useful in correlation between Olduvai Gorge, the type area of the Olduvai Normal Palaeomagnetic Event, and other sequences. As more palaeomagnetic studies are carried out on the fossiliferous lacustrine sediments of the East Rudolf area (and also other parts of the Kenya Rift) a pattern of epochs and events within the geomagnetic time-scale is being recognized. This supplements the potassium-argon determinations in dating important hominid and artefact finds³⁸⁻⁴⁰.

(d) Uranium series. Methods based on the radioactive daughter products of uranium have been put to extensive use in dating only recently and the problems are not yet as well understood as for ^{14}C . Three methods have been applied. Decay of ^{231}Pa - ^{230}Th has a range from 0 to 300,000 yr and is applicable only to marine sediments. The relationships of ^{234}U - ^{231}Pa - ^{230}Th have a range of 0 to 150,000 yr or in some cases a little older. Helium-uranium dating of corals raises several problems but seems reliable in the range 0 to 100,000 yr⁶. The materials suitable for the methods are coral (unrecrystallized and cleaned aragonite), evaporites and volcanic rocks containing uranium; clean unaltered inorganic carbonates (tufas, travertines, colites).

Mollusca, bones and other fossils are often rendered unsuitable because of alteration. In view of the age limitations and the fact that coral is so far the only material which has been extensively tested, the method has only indirect application to continental environments. Nevertheless, a date has been published for fossil bone samples from the Isimila Acheulian locality, Tanzania⁴¹. If the method can indeed give meaningful results for continental deposits in the time-span older than 50,000 yr this could be a most important development. Worries remain concerning the "open" nature of bone systems, however.

The problems of radiometrically dating the episodes of high sea level and shorelines of last interglacial age, and also deposits of deep-sea cores assigned to the same period on palaeotemperature evidence, have been discussed by Shackleton⁴² following work by various authors (refs 43 and 44, for example). Most of this sea-level evidence depends for calibration on uranium series dates on coral and oolite. If a firm pattern of dates could be established for the last interglacial there would be the possibility of a link with continental deposits assigned on palaeoclimatic evidence to this period. But reservations expressed by Thurber, Broecker, Bender^{5,6} and others who have undertaken this research and the conclusions of Shackleton⁴⁵ suggest that firm isotopic dating is not yet available for marine successions of last interglacial age. Shackleton⁴⁵ further comments that "many terrestrial Pleistocene deposits are ascribed to well-known named stages on the basis of an inadequate model" and thus he is "loath to comment on age determinations which are construed as dating these stages".

From a consideration of the radiometric data for the age of palaeotemperature peak 5e in the deep sea cores, Shackleton⁴² suggests that it lies between 100,000 and 130,000 yr BP. He concludes that the radiometric dates "do not show sufficient agreement to distinguish between the possible ages predicted" by various versions of the Milankovitch hypothesis and that "Workers interested in using the Milankovitch hypothesis as a framework for dating events further back in the Pleistocene can draw no comfort from the ease with which the hypothesis may be modified"⁴².

(e) Fission track. The techniques and problems associated

with this method have been summarized recently by Fleischer and Hart⁴⁶. The method depends on the detection in minerals and glasses of trails of intense damage following fission of a uranium nucleus. As the major isotope of uranium (²³⁸U) decays at a slow and steady rate it is only necessary to establish the fraction of uranium atoms that have fissioned within a given sample in order to compute its age. The age range of the method is from 20 to 5×10^9 yr and so it has potential as a means of cross-checking the results of several isotopic methods already described. Indeed it was used successfully as a broad check on the initial potassium-argon ages established for Bed I at Olduvai Gorge⁴⁷ and has also been used at various recent archaeological sites and to date falls of tectites. Materials that can be dated include uranium-rich accessory minerals (apatite, zircon, sphene, and so on) from tuffs, igneous or metamorphic rocks. Obsidian, pumice and heated rocks or archaeological implements can be dated to determine their time of heating. Study of the tracks is made possible by etching out the lines of damage and a large variety of etchants has been used⁴⁶. The method has application to the past 5 m.y. but more data are now required to broaden the coverage.

Cerberus—the Dating Dog

Having reviewed the methods that have contributed dates relevant to the calibration of a time-scale for human evolution, it is important to stress the necessity of synthesizing information which for convenience may have been gathered and processed under the separate heads of chronometry, stratigraphy and biometry. The central need for sound stratigraphic control makes it possible to portray geochronology by reference to "Cerberus", the mythical three-headed dog (Table 1).

The principal subdivisions are summarized under the three heads and it is a basic requirement that Cerberus should have a pedigree (A1 to A4 as detailed above) in common with all other dogs. It is essential for information to be fed through and exchanged between any of the three heads. Finally, it is not entirely frivolous to add that although a climatic "tail" may occasionally have its part to play in maintaining the wellbeing of the whole animal it should never be allowed to "wag the dating dog".

Time-scales—Long and Short

Evans⁷ has recently used climatic cycles as if their duration was reliably established and each could with certainty be related to observed features in the stratigraphic column. He derived a time-scale based on twenty-five alternately warm and cold cycles each averaging 40,000 yr duration commencing from the "Calabrian" dated at 1 m.y. ago. In a summary chart significant "radiometric dates tied to climatic phases" are related to "insolation and climatic cycles" and also to river terrace heights in Britain, to Mediterranean high sea

levels, to magnetic stratigraphy and to "approximate dates of cultures" and fossil hominid finds.

This situation seems remarkably similar to that developed in East Africa some years ago when a theoretical climatic-stratigraphic sequence was in vogue. In Evans's system twenty-five warm and cold "pigeon holes" have climatic and other events slotted into them. In much the same way archaeological and geological data were placed into the pluvial and interpluvial compartments of what proved to be the East African "climatic straitjacket". Fortunately in East Africa theoretical climatic considerations have been rejected in favour of normal stratigraphic practice⁴⁸⁻⁵⁰. Firm evidence of climatic parameters is now being calibrated by isotopic dates and a pattern of climatic change is gradually emerging^{21,51}.

Problems of calibrating even the last interglacial satisfactorily have already been touched on under (a). Recent work by Imbrie and Kipp⁵² attempted to quantify climatological data for the late Pleistocene obtained from micro-palaeontological investigation of deep sea cores. No simple "fit" with astronomically predicted climatic change was found. But if such an approach can be combined with rigorous calibration and extended to many more cores it should enable the validity of climatic hypotheses to be fully tested.

Dating glacial tills for periods before 2.0 m.y. has been successfully undertaken in the Sierra Nevada of North America⁵³, in Southern Argentina⁵⁴ and elsewhere, by combined application of potassium-argon dating and palaeomagnetic studies. Again a broad pattern of climatic rhythm seems likely to emerge as more dates accumulate.

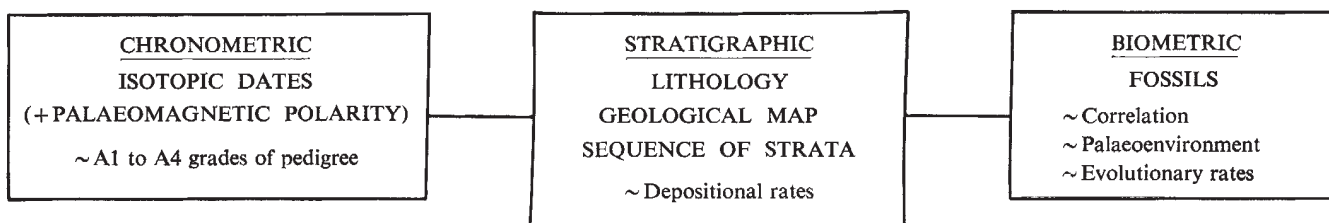
For marine successions it has been fashionable to talk of "short" and "long" chronologies. Cooke has set out the details of each and the problems of reconciling them⁵⁵. The "short" scale originated from oxygen-isotope curves constructed by Emiliani⁵⁶; is calibrated by extrapolation beyond the range of ¹⁴C dates by reference to sedimentation rates; receives some support from uranium-series dates for the last interglacial; and has been extended to European continental sequences.

The "long" scale was introduced by Ericson, Ewing and Wollin⁵⁷; has been modified by various workers as summarized by Berggren⁵⁸; is largely in accord with the geomagnetic polarity scale; and allows correlation with continental deposits in East Africa calibrated by ⁴⁰K/⁴⁰Ar for the age range from 1 to 3.0 m.y. and older.

As far as the hominid record is concerned a longer scale seems to be demanded as artefacts and fossils from well-dated contexts range back through 2.0 m.y. to beyond 2.6 m.y. This seems impossible to reconcile with short scales where Acheulian or bifacial artefacts only range back to about 350,000 yr.

In fact there remain many gaps in the evidence. The South African caves containing australopithecine-bearing breccias together with the Choukoutien caves which yielded the Pekin *Homo erectus* material are undated by isotopic methods. The *Homo erectus* open sites in Java have some

Table 1 Cerberus, the Dating Dog*



* A feedback mechanism exchanges data between all three heads and the pedigree similarly reflects information from all sources.

radiometric control⁵⁹ but lack the clearly mapped long sequences of strata and fossils of the Kenya rift localities. Even in East Africa enormous blanks remain, particularly between about 1.5 m.y. and 50,000 yr BP, for which more fossil evidence has still to be found or isotopic dates have yet to be established¹⁰. Judging from the abundant finds and dates recorded during the past ten years, rates of human evolution should be discernible during the next decade.

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Future of Electron Microscopy in Biology

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Electron microscopy, at present a versatile tool in the study of biological systems, can benefit from advances in instrument technology and its application.

Of all the methods now available for studying matter through its interaction with radiation, electron microscopy is the most versatile and the least specific. Nearly all the advanced techniques presently available require the presence of many identical units in the specimen. These techniques are immensely important to modern chemistry, and, indeed, methods such as X-ray crystallography already play a vital part in molecular biology; but their applications to the highly complex and

irregular structures encountered in biology must be limited. Electron beam technology is, however, unique in providing useful information down to the molecular and atomic level about the most complex and random of structures. Hence special importance for biology. A central theme of this paper is the assessment of how one may hope to increase the precision of the information that electron beam technology can provide about systems which by the standards of the physical sciences are highly intractable.

A typical unicellular organism, less than 2 μm across, synthesizes many hundreds of compounds by a precisely regulated process, is able to replicate itself, and is capable of the genetic evolution and modification of these processes. If one wanted a quick way of destroying that uniquely delicate mechanism there is probably no better tool than a flux of fast electrons. Yet precise information can be obtained about a

meticulously prepared biological specimen by exposing it to a beam of electrons which in one second would boil away many times its weight of water.

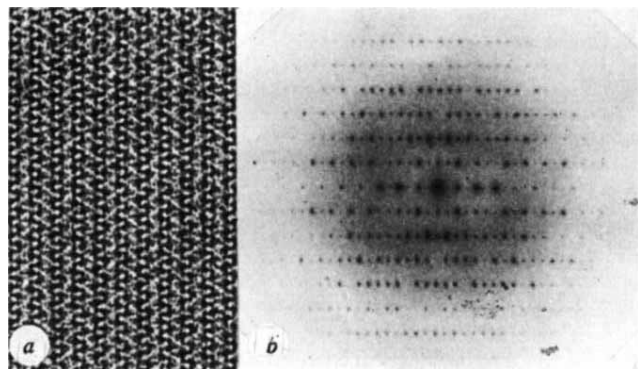


Fig. 1 *a*, Beef liver catalase ($\times 175,000$) treated with equimolar solutions of aluminium and uranyl formate and imaged over a hole in the substrate with a minimal electron dose. *b*, Optical diffraction pattern of the same area ($1\text{ cm} = \frac{1}{25}\text{ nm}^{-1}$).

When electrons interact with matter they may pass through the specimen undisturbed; they may be elastically scattered by atomic nuclei with little loss of energy but with a wide spatial distribution, or they may interact with orbital electrons, being scattered with substantial transfer of energy but with small deflexion. Electron microscopy aims at interpreting and presenting as much as possible of the information about the morphology of the specimen which is carried by the scattered electrons. The consequences of the transfer of energy to the specimen are also of fundamental importance. Transitions of electrons between quantum levels may be accompanied by characteristic X radiation carrying analytical information. Electrons may be ejected with the formation of highly reactive species whose subsequent reactions change the chemical composition of the specimen, one of the most important causes of radiation damage. All such phenomena are inevitable consequences of exposing a specimen to high energy electrons, which initiates processes which can yield morphological and analytical information but also leads to reactions which alter its nature. The art of electron beam technology is to exploit these benefits while minimizing the deleterious effects.

Beam Technology and Biological Materials

The wavelength associated with a 100 kV beam of electrons is about 0.004 nm, so that the theoretical resolving power is far beyond what is needed to image any specimen, biological or mineral. In practice other limitations arise, and the lowest level at which one can get a reliable point-to-point image in the most favourable circumstance is about 0.3 nm. Strenuous (and costly) efforts are being made to achieve resolutions down to 0.1 nm, which, if there were no other problems, would permit the direct visualization of atoms in biological materials—perhaps the ultimate in ultrastructural investigations.

Instruments are now commercially available which can resolve distances of 0.5 nm or less. Direct visualization of biological material at this level would tell us much about the structure and mode of action of macromolecules and sub-cellular components such as contractile proteins and ribosomes, the nature of cellular interfaces, the ultrastructure of viruses, and about much else that can now only be inferred from biological and biophysical investigations, and is at present a matter of controversy and conjecture. Biologists would like to be able to work at these resolutions in circumstances where they can be sure that they are studying the specimens in their natural state. Unfortunately, serious problems stand in the way.

One of these, radiation damage, has already been briefly mentioned. As it invariably accompanies the process of observation, the object is to minimize it while at the same time getting as much information as possible. No biological specimen will remain entirely intact during observation: all one can hope for is to observe an entity which closely resembles the original specimen, and not a completely different artefact. In fact radiation damage can rarely be identified in biological specimens at resolutions higher than 1 nm, and it is a real possibility that deterioration may be virtually instantaneous, so it is to be hoped that the original structures survive substantially unchanged in the degraded product actually observed.

Although too little work has been done on the direct investigation of beam damage to biological specimens *in situ*, a good deal is known about radiation chemistry that is relevant to it. Much of this work has involved thin specimens, seeking model systems for the study of biological macromolecules among amino acids, polypeptides and carbon-chain synthetic polymers. Radiation damage appears to be diminished at low temperatures, perhaps due to the trapping or inactivation of radicals. It has been reported that damage results primarily from interaction with water in the residual gas in the instrument, though electron diffraction patterns of proteins have been obtained in environmental cells at a relative humidity of over 90%. There is a strong suggestion that high operating voltages favour survival: thus in one experiment using the high voltage electron microscope, damage to a crystalline synthetic polymer was found to decrease by a factor of 10 when the operating voltage increased from 50 kV to 1 MV. Supporting evidence has come from studies of amino acids between 100 kV and 2.5 MV.

To minimize radiation damage, as low a flux of electrons as will give an image unimpaired by statistical fluctuations in the beam must be used and there could be room for improvement in this direction in the practice of biological electron microscopy. Another approach, at present a matter of controversy, is the use of image intensifiers.

Other mechanisms of damage arise in the study of thick sections; particularly the knock-on effect by which an atom is ejected bodily from a molecule by an incident electron. If the specimen is sufficiently thick the displaced atom may be sufficiently energetic to displace another, and this sets up a chain of disruption until the initial input of energy is sufficiently dissipated. This effect becomes increasingly important above the threshold beam voltage above which an electron is sufficiently energetic to displace an atom (in the case of carbon, 30 kV). At still higher voltages (100 kV in the case of carbon) it begins to fall off again. It is another factor to be taken into account in determining the optimum conditions for operation.

Another problem, of comparable importance for the biologist to radiation damage, is that of obtaining adequate contrast in the specimen. The biologist is nearly always concerned with light elements of low scattering power. At the levels of resolution necessary for the study of cellular and sub-cellular particles, the most important method of increasing contrast is to observe not the specimen in the original condition but an object containing heavy metal atoms of high density. Three approaches to this have been evolved: (i) positive staining—the direct reaction of the specimen with heavy metal compounds; (ii) negative staining—replacing the hydrated regions of the specimen with electron dense material; (iii) shadowing—depositing an electron dense substance (usually a metal) onto the surface of a specimen at an oblique angle to accentuate its surface features. Some compromise is involved in all these methods, because to make the specimen visible it must be modified; here again, there is a danger of observing no more than an artefact. Specimen preparation is as important here as in other aspects of biological electron microscopy.

Staining is still largely an empirical procedure lagging behind development of instrumentation. Accurate methods are, however, being developed based on principles capable of wider application. In enzyme histochemistry our knowledge of

the basis of reactions at specific substrate sites is comparatively well advanced. Uranyl acetate staining of DNA has been used to visualize aggregates of a few atoms in a strand about 3 nm thick. Very substantial rewards could come from the development of specific stains which can be applied so lightly that they do not appreciably modify the specimen—for instance the direct determination of the base sequence of DNA. The solution of such problems will demand all the resources of modern quantitative metal ligand theory. Negative staining may also be put on a more rational physicochemical foundation. The interaction between polyanionic species and biological specimens is found to be very sensitive to the condition of the aqueous medium, and unexpected analogies discovered between their solution chemistry and that of simple ionic species, which may contribute to our understanding of their action. Uranyl salts have been widely used as negative stains, but deteriorate during irradiation due to crystallization. Recent work has demonstrated the advantages of mixed systems over pure uranyl salts.

Investigation of Soft Tissues

What must be done before a soft tissue, consisting mostly of water, can be studied in a microscope? Most of the current methods for fixing and embedding are unspecific. Although they have proved more effective in preserving biological structures than might have been expected, they must also involve considerable distortion of the specimens, so inferential evidence from light microscopy, biochemistry and biophysics must be used to support observations. There is room for an organized physicochemical attack on these problems, but the best way of overcoming them is to avoid them altogether, for example, by so modifying the instruments that they observe the specimen in its natural environment—we return to this later. The alternative is to immobilize the water in the specimen by freezing.

The study of frozen tissues in the electron microscope is theoretically feasible, but formidably difficult in practice. Much modern work is concerned with developing and applying methods of very rapid cooling, which is essential because the ice crystals formed must be sufficiently small as not to interfere with the electron image, and because when the ice crystals are large, and an appreciable amount of solute is present, high local concentrations of ions can arise which might damage the specimen by osmotic shock. One method being developed operates at high pressures to give very small crystals at depths up to 200 μm in the absence of cryoprotective. In another, droplets of the specimen of diameter about 10 μm are sprayed onto liquid propane at -190°C . There has been considerable discussion as to whether truly vitrified water can be produced by the rapid freezing of specimens. This matter is still controversial.

The preparation and handling of frozen specimens present their own problems. If the specimen is warmed above the glass transition point of ice (-130°C) irruptive ice crystal growth may occur. Frozen tissues may be studied intact, but work with thin slices promises more. Ultracryomicrotomy is already an established technique, and several commercial instruments are available which can cut sections down to 20 to 40 nm. At present the lower limit attainable in usable sections is about 40 nm. To get thinner sections more needs to be known about the actual physical processes involved when cutting a frozen section with a microtome. The ideal temperature for section cutting is still doubtful. Ice crystal growth must be avoided, and we are still uncertain whether the process of cutting causes localized melting. Problems also arise in the transfer of the specimen. Devices are now being developed which avoid dehydration of the specimen, or its contamination by ice condensed from atmospheric vapour.

These difficulties apart, other problems arise with frozen sections. It is not yet certain that large quantities of water do

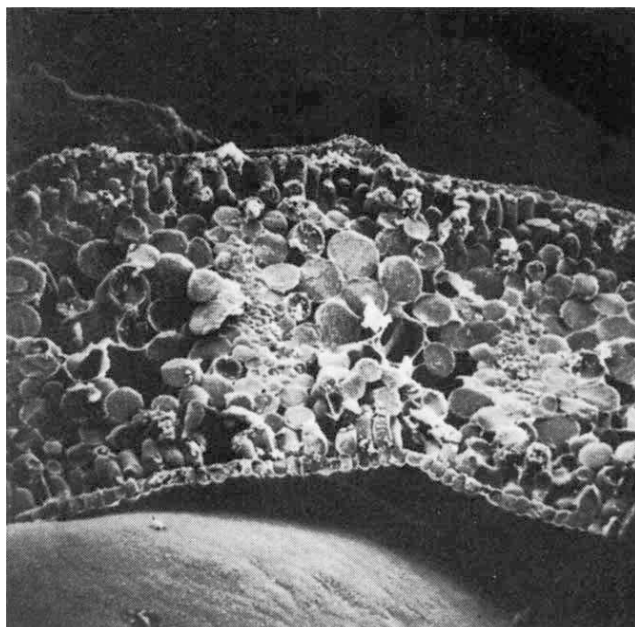


Fig. 2 Cryofractured *Muscari botryoides* leaf. The leaf was quench frozen in Freon 22 at -165°C and fractured under the cryogen. The specimen was then kept frost free at -130°C and coated with 15 nm of evaporated gold-palladium. The still-frozen leaf was transferred to the cold stage of a 'Stereoscan' S4 scanning microscope and photographed at -150°C at an accelerating voltage of 20 kV. Magnification $\times 200$.

not cause excessive electron scattering, with a consequent fall in signal/noise ratio, and it may prove necessary to compromise by removing free water from specimens for cryobiological study. It is not yet entirely certain that the electron beam does not cause melting: if it did, it would completely nullify the aims of the cryobiological approach. On the other hand, there may be incidental advantages. Radiation damage may be lower at cryobiological temperatures and cryobiology will certainly stimulate ultrahigh vacuum technology. Vacua better than 10^{-8} N m^{-2} (10^{-8} torr) will become increasingly important in reducing contamination in ultrahigh resolution freeze-etching studies, which aim to provide more information about the process of fracture in relation to the contours of the specimen.

Environmental cells would avoid all these problems, but they introduce their own difficulties. In the past much emphasis has been placed on the preservation of vitality during exposure to the electron beam, but the comment that work in this direction "shows a healthy disregard for theoretical pessimism" seems well justified. Their use in the direct investigation of hydrated specimens is more promising, but also introduces its own problems, for the interaction of the electron beam with water vapour produces high energy ions which can be very destructive. Detailed investigations will be necessary to overcome the problems of instrument design imposed by the use of environmental cells, and for the assessment of their value in biology.

High Resolution Microscopy

Here we consider high resolution microscopy separately on two counts: (i) different mechanisms of imaging and of contrast formation are predominant at high resolutions, and (ii) the highest resolutions are primarily of importance to the biologist in the study of macromolecules (including viruses). (For investigations at the cellular and subcellular level, of course, problems of specimen preparation and handling are the limiting factors and the highest resolutions are unnecessary.)

There are strong incentives within the physical as well as the biological sciences for the development of high resolution instruments, and advances are likely to come from the convergence of improvements in design now being sought in many programmes rather than from any radically novel departure (though the scanning transmission electron microscope permits a substantially novel approach to high resolution). Whereas at relatively low resolutions the electron image is formed primarily by amplitude contrast, which is the consequence of the scattering of electrons by the specimen, phase effects predominate at high resolutions. Phase changes introduced into the electron beam by the specimen combine with phase changes introduced into the incident beam by the objective lens of the instrument to give an amplitude contrast at the imaging plane. The attainable resolution is limited by spherical aberration effects in the objective lens. Much high resolution technology is directed towards limiting its effects, for instance by use of quadrupole and octopole lenses, or by the use of high frequency currents to energize the electron lens. Alternatively one can accept the consequences of spherical aberration, but seek to isolate them from the useful information carried in the electron beam by introducing a suitably designed and located zone plate. Calculations indicate that at 200 kV, zone plates could increase resolution at the 0.2 nm level by about 0.05 nm. A different approach makes use of the fact that all the useful information about the specimen is contained in the image, even though it is masked by spherical aberration and other effects. Knowing the characteristics of the lens system the information recorded on the photographic plate can be translated into digital form and computer techniques used to sort out the useful information. Computing equipment of high capacity is needed at relatively low levels of accuracy.

High resolution microscopy is also limited by chromatic aberration of the lens. In principle this can be minimized by suitable design of the electron lens (the use of superconducting lenses has been mooted in this connexion), but it is unavoidable in heterogeneous specimens of appreciable thickness, such as are often necessary in biological specimens if contrast is to be adequate. Several approaches are being made to improved contrast. The use of low voltages is being explored, but is open to many of the objections that are leading into work at increasingly high voltages. The use of phase plates may be more promising: a suitably designed plate can enhance contrast without significant loss of resolution. Strioscopic methods are also of potential value for high resolution work.

High resolutions demand very accurate instrumentation and are inherently costly. They demand very stable beam voltages and lens currents and high mechanical stability. Problems of preparing and handling the minute specimens which are necessary are especially acute, and the need for extremely thin support substrates may be another limiting factor.

A different approach to high resolution uses scanning electron microscopy, in which a fine beam of electrons is traversed point by point across the specimen, allowing the detail of the interaction of the electron beam with each part of the specimen to be observed separately. This contrasts with the conventional microscope in which the whole area of the specimen is exposed to the electron beam, and all points of the image are generated simultaneously on the viewing screen. Scanning instruments are already well established in which signals produced by reflected electrons, X rays, or light are used to build up an image of the surface of the specimen. Recently, however, the availability of very bright electron sources has made possible the development of scanning instruments operating at high resolution in the transmissive mode. With such instruments the number of transmitted electrons of any particular category can be isolated and counted separately for each point of the specimen. This can yield much information about the characteristics of individual points—for instance, the ratio of inelastically to elastically scattered electrons is directly accessible, and provides a means for measuring the average atomic number of the material excited by the probe. As the

scanning transmission microscope involves no lens between specimen and detector, restrictions on its accuracy due to chromatic aberration do not arise, and the resolution is limited only by spherical aberration, diffraction effects, and by the size of the probe. Probe diameters of 0.5 nm have already been achieved with field emission guns.

To visualize individual biological molecules higher voltages than are now used may be necessary: the range between 100 kV and 500 kV has been suggested as a compromise between theoretical requirements and the need to avoid gross radiation damage, though still higher voltages may be practicable. The hypothetical system using a zone plate would, it has been suggested, be just capable under ideal conditions of imaging labelled bases of short strands of polynucleotides in unstained specimens, but greater hopes are held out for the study of enzyme substructure, and the visualization of active sites labelled by heavy atoms.

High Voltage Electron Microscopy

The chief incentive for working at high voltages is the greater depth of specimen preparation that they allow, which with biological materials is nearly proportional to the applied voltage. This increase in penetration makes it possible to investigate the three-dimensional ultrastructure of chromosomes in specimens 1.5 μm thick. Under favourable conditions even thicker sections have been studied.

The theoretical resolving power advantage of an electron microscope is nullified in practice by problems of electrical and (particularly) mechanical stability. Contrast falls off rapidly with increasing voltage, so that image intensification techniques may be necessary to give a useful picture of thick specimens. The large depth of focus means that a great deal of information is projected on the two-dimensional viewing screen, and special methods of image analysis may be necessary to obtain a comprehensible picture.

The most desirable operating voltage is still a matter of doubt. Several instruments have been built in the range 1 MV and above, but it has been proposed that the range up to 500 kV, within which chromatic aberration effects already show a substantial improvement, should be explored. Economic considerations are important here: a rough guide is that in the high voltage range the cost of the instrument is proportional to the operating voltage.

Image Analysis

The almost universal practice in electron microscopy is to preserve morphological information in the form of amplitude contrast in the electron beam, and to observe the image on a fluorescent screen or record it on storage cathode ray tubes, magnetic tape or photographic plates. Methods are being devised by which information is directly converted to digitalized form in which it can be processed without photographic recording, but photographic plates are at present the most efficient recording technique and are used at every level of resolution. A recurrent problem in the study of all but the thinnest specimens is that information about a three-dimensional object is recorded as an image in two dimensions. As a conventional instrument has a depth of focus of at least several hundred nm, this means that the final image is a superposition of all the features along the direction of the beam within its depth of focus, without any perspective effect. Some way must therefore be found of extracting the contribution from different levels in the specimen.

When working at comparatively low resolutions it is possible to cut several thin sections, observe them as two-dimensional objects and combine the serial images to give a three-dimensional representation of the specimen. Alternatively stereomicrographs can be produced from pairs of pictures of the

same area of the specimen, inclined to each other at a suitable angle; this is especially useful in high voltage electron microscopy. Recently, direct stereoviewing has been developed for use in the scanning microscope.

To acquire more detailed information about structures more sophisticated methods are necessary. All the information extracted from the specimen by the electron beam is preserved in the electron micrograph: the problem is to extract and interpret it. The amplitude contrast information contained in the optical diffraction pattern of the electron micrograph can give information about periodicities in the object (and can, incidentally, "clean up" the image by eliminating background caused by specimen irregularities and present information not readily detectable by eye). For greater detail phase contrast information, which is in principle accessible by holographic methods, must be used; although these have been mooted for many years they are only now showing promise of being a practicable proposition. More has been achieved from a digitized array of optical densities of the image obtained by microdensitometry. By analysing a (calculable) number of planar projections it is possible to reconstruct the three-dimensional image. The number of projections needed depends on the degree of symmetry of the object, and most work has been done with objects such as negatively stained spherical viruses, where a few projections are sufficient to resolve a particle of about 20 nm diameter to 1 nm, the limit of resolution attainable with negative staining. For substances with helical symmetry such as muscle filaments sufficient information for structural analysis can be obtained without measuring or analysing phases. Although the amount of computation necessary for objects of low symmetry is great, there is no reason in principle why this method should not be applied to any structure. An acceptable micrograph contains about 10^8 picture elements and can record up to twenty grey levels (a commercial television operates with five). Rapid computational techniques have been developed which make the processing of information at this level of detail a practical proposition, and it has been proposed that the necessary computing equipment should be made integral with the electron microscope. With improved methods of specimen preparation and preservation, and with the use of faster computers of higher capacity, the three-dimensional analysis of biological structures, such as ribosomes, at still higher resolutions should be a practicable proposition.

Microanalysis

When a high energy electron beam, such as is used in conventional transmission and scanning electron microscopy, interacts with matter, characteristic X rays are produced. If the beam can be brought to a fine focus it affords a means of analysing a very small surface area, for X radiation from atoms, even of closely similar atomic number, can, in principle, be sharply resolved and accurately quantified in a spectrometer.

In practice the efficiency of X-ray microanalysis is limited by background noise in the signal (especially important in biological materials), the elements of interest are usually at low concentrations) and by the measuring efficiency of the detector. X-ray detectors are of two kinds—wavelength diffracting spectrometers and energy-selective analysers. Crystal diffractometers are capable of accurate analysis, but their collecting power is limited by basic optical considerations and by problems of design. Energy analysers have low quantum resolution, and their response to monochromatic radiation takes the form of electrical impulses distributed about a mean amplitude, which, especially with light elements of biological interest, are vulnerable to interference from background radiation. On the other hand, they are capable of much higher collecting powers than are crystal diffractometers.

With currently available detectors the minimum amount of an element in a biological tissue that is measurable by X-ray

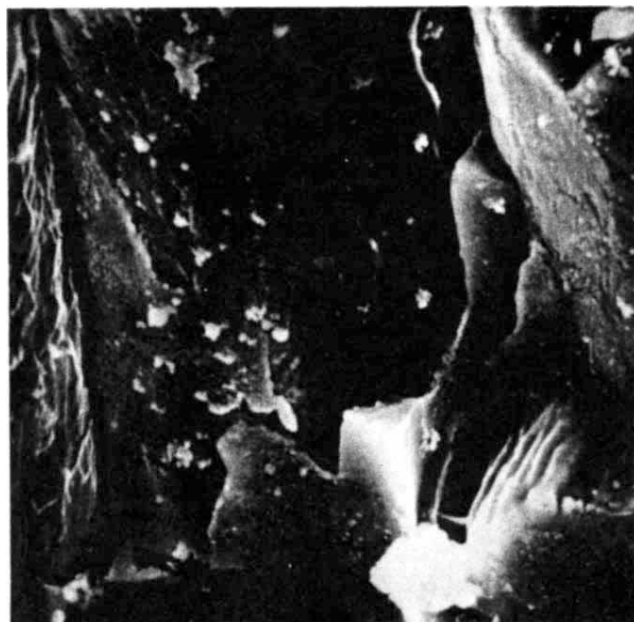


Fig. 3 Cryofractured *Vicia faba* root ($\times 2,000$) which has been treated in the same way as described in Fig. 2. The cryofractured leaf in Fig. 2 shows the correct orientation and juxtaposition of the cells and demonstrates that the watery cell contents are still present, but in the frozen state. The root picture here shows part of the wall torn off revealing the underlying cytoplasm. Part of the intercellular space, which would be filled with liquid in the living state, can also be seen.

microanalytical methods is about 10^{-18} g and the minimum weight/weight ratio (in the volume exposed to the microprobe) about 100 p.p.m. This puts a lower limit on the size of specimen that can be exposed to a probe and give a useful signal. The spatial resolution of X-ray analysis is also restricted by the spread of excitation in a (thick) specimen exposed to the beam: in typical operating conditions a probe 1 μm in diameter will excite a volume of about 5 μm depth and diameter. This difficulty can to some extent be mitigated by working with specimens of thickness between 0.5 and 5 μm , when excitation will occur with a cone 1 μm or less in thickness. With very thin sections (150 nm or below) still higher resolutions can be achieved, but at the expense of now exciting a small volume and producing a small flux of X radiation.

This discussion relates to probes formed from the hot tungsten filament electron emitter which until recently was invariably used in electron beam technology. They have an inherent maximum brightness, so that a spot size of 10 nm is needed to give an image in a reasonable time. Recently brighter sources have been developed. Sources using rare-earth borides are two or three orders of magnitude brighter than tungsten filaments and the field emission gun is several orders brighter still. It should make a spot size of 2 nm diameter a feasible proposition, but the current at present attainable in so small an area is likely to prove insufficient for microanalysis except perhaps for high elemental concentrations.

An important typical biological application of microanalysis is the determination of the distribution and movement of ions and other species in cells and tissues. Currently available technology should allow ion concentrations within organelles, between different parts of the cytoplasm and across cell boundaries to be measured. Resolutions an order of magnitude better might lead to better measurements of concentration gradients across individual membranes and even within the membrane itself. This could provide important evidence to moderate between current rival theories of the mechanism of membrane action.

As in other biological applications of electron beam technology, problems of specimen preparation predominate in

microanalytical investigations of this kind. The basic problem is to ensure that the intracellular ions being studied stay where they were in the living cell throughout the preparative procedures. It seems at present that the only promising technique involves the use of frozen sections, with the inherent difficulties of preparation and handling.

Progress in the application of electron microscopy to biological problems depends both on advances in instrument technology and on knowledge of how to apply those advances to the study of biological systems. At present the latter lags behind, and much of the potential of electron beam technology is frustrated because the biologist cannot yet use it. Work is especially urgently needed in studies of the radiation damage of biological materials and of methods of specimen preparation and manipulation. This will involve the interaction of a wide range of scientific disciplines.

Legacy of van der Waals

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It is a hundred years since van der Waals related the pressure, volume and temperature of a fluid by the equation which bears his name. This article, which is based on a lecture delivered at an Institute of Physics Conference at the University of Kent in April, describes the background and present value of his work.

JOHANNES DIDERIK VAN DER WAALS never learned Latin and so was barred from the regular university course in his home town of Leiden. He studied part time, but it was only after new legislation that he was able to present a thesis in June 1873, at the age of thirty-five. Three years later he was offered the chair of physics in the new University of Amsterdam, where he lived until his death in 1923.

His thesis¹⁻⁵ and more than a hundred papers which followed it dominated the study of liquids until 1914. There was then a rapid loss of interest until he was remembered only for the equation of state which had been the climax of his thesis. The purpose of this article is, first, to put what he wrote in the context of its day and, second, to examine the ideas which have flowed from his work and to show why there has been recently such a revival of interest in it.

His Predecessors

By 1873 physicists had substantially correct ideas of molecular speeds, masses and sizes^{6,7}. Clausius, Maxwell and Boltzmann had tackled, with some success, both the equilibrium and transport properties of the dilute gas, but even the equilibrium theory of denser fluids was still in a primitive state. It was known that gases other than hydrogen were more compressible than Boyle's law required, that this compressibility was due to intermolecular forces stronger but of shorter range than gravitation, and that it was the space occupied by the molecules which made hydrogen a "fluide élastique plus que parfait"⁸. In the eighteenth century⁹ Bernoulli had proposed that in Boyle's law the specific volume v be replaced by $(v-b)$, where b was thought to be the volume of the molecules.

The principal directions of development of biological electron microscopy are towards structural studies at higher resolutions and the development of accurate analytical techniques. The immediate biological applications of the former fall primarily in the field of molecular biology. Analytical techniques are likely to prove very important in studies at the cellular and subcellular level.

Much also remains to be done in the use of established instruments in biological work, and improvements in their performance and design, and the development of ancillary equipment, will all extend significantly the scope of the types of instrument already established.

Figure 1 was taken by Dr Nigel Unwin at the MRC Laboratory of Molecular Biology, Cambridge. This paper is a shortened version of a report commissioned by the Biological Sciences Committee of the Science Research Council.

Hirn^{10,11} (1863), Dupré¹² (1869) and others revived this proposal, but van der Waals tackled the problem anew by calculating the effect of molecular size on the mean free path. He thus showed that the covolume b (the name is due to Dupré (ref. 12, page 61; see also ref. 13)) is four times the volume of the molecules, although he realized that the substitution of $(v-b)$ is valid only at low densities, that is, at the level of the second virial coefficient in modern language. (Rayleigh^{14,15} was apparently the first to see that the substitution of $(v-b)$, when b is the volume of the molecules, is exact in one dimension at all densities.) Later he tried to calculate the covolume for denser fluids but his results had to be corrected by Boltzmann and van Laar¹⁶. This problem is still unsolved, and could not even be tackled systematically until the development of graphical expansions between 1920 and 1940.

His calculation of the effect of the attractive forces on the pressure was more fruitful. Young and Laplace had known that these were responsible for the surface tension of a liquid. The latter showed that the pressure in a drop of radius l could be written $(K+H/l)$, where K and H are integrals, over the separation r , of the mutual force between two elements of liquid. He put the density equal to "unity", but those who followed took the force to be proportional to the mass in each volume element, and so multiplied his results by the square of the density. Laplace's modified results, obtained both by Dupré, helped by his colleague F. J. D. Massieu (ref. 12, pages 147, 150-157), and by van der Waals, can be written in modern notation by supposing there to be a potential $u(r) \leq 0$ between each pair of N molecules in a volume V

$$K = -\frac{1}{2}(N/V)^2 \int u(r) dr \quad H = -\frac{1}{4}(N/V)^2 \int ru(r) dr \quad (1)$$

(Before about 1880 there was a casual attitude to units and dimensions which can make it difficult to decide what was meant. This difficulty probably led to Rayleigh's mistaken assertion¹⁷ that Dupré found K to be proportional to density. van der Waals sometimes omits the factor of the square of the density.)

Young estimated K at several thousand atmospheres, a result which Dupré confirmed by showing that it is $(\partial U/\partial V)_T$ in a liquid, where U is the *travail de désagrégation totale*, or configurational energy. Since U is zero at zero density

$$U = -KV = \frac{1}{2}(N^2/V) \int u(r) dr \quad (2)$$

and so, by purely thermodynamic argument

$$Tf(V) = p + K \text{ or } p + a/v^2 \quad (3)$$

Dupré obtained equation (2) for a liquid in his book, but not equation (3). He died in 1869, the year it was published. Others had, without such thorough discussion, also proposed an internal pressure proportional to the square of the density¹⁸⁻²¹.

In 1863 Hirn^{10,11} had written

$$(p + \Phi)(v - b) = RT \quad (4)$$

but although he realized that Φ greatly exceeded p in a liquid, and that it might be a function of volume only, he never wrote it as a/v^2 .

van der Waals's Equation

van der Waals proposed

$$(p + a/v^2)(v - b) = RT \quad (5)$$

He did not use his derivation of Laplace's K to obtain directly the term a/v^2 , but justified the latter by a simple argument repeated today in most textbooks. Only towards the end of the thesis did he tentatively identify a/v^2 with K , at least for carbon dioxide, but he followed this by other less useful relations between K and the equation of state, which he omitted when the thesis was translated (ref. 1, pages 97-99).

At first sight he seems to have done no more than to combine some current ideas. Why then did his work have so great an influence? First, because his ideas were clearer than those of his predecessors. He understood, as they did not, the new kinetic theory of gases, and so was able to obtain the correct covolume. He used Clausius's virial theorem²² of 1870, together with Laplace's static view of matter, in a way which appealed to his contemporaries even if it did not always convince them.

Second, his work came soon after the discovery of the continuity of the gas and liquid states by Andrews^{23,24}. Hirn and Dupré had tried to represent only the pressure of gases; they discussed liquids also but could not, before 1869, have aspired to fit both states with one equation. This van der Waals did, and the influence of Andrews is unmistakable—his title is a translation of that of the Bakerian lecture²³.

His equation is a cubic in v and so has either one or three roots for a given p and T . There is one if the temperature exceeds the critical; that is, if $RT > RT^c = 8a/27b$. There are three at lower temperatures, the smallest and largest of which are liquid and gas, but not necessarily in their saturated states. Thus the equation represents both Andrews's continuity of state and the sigmoid loops which his colleague James Thomson had already proposed as a continuous isotherm in the two-phase region²⁵.

Although written in Dutch, the thesis received immediate attention in Britain. Andrews called on one of his daughters to help, and in February 1874 wrote to Thomson²⁶ from Belfast that "Bessy and I are hard at work on Diderik van der Waals!" Maxwell first noticed it publicly in June 1874 in a review^{27,28} of a book by Plateau, whose macroscopic approach he contrasted with the molecular picture of van der Waals. In October he reviewed the thesis^{29,30} favourably, and next year showed how the saturated volumes and the vapour pressure p_0 could be obtained from the sigmoid isotherms³¹⁻³³. His construction was the "equal-area" rule; the line $p = p_0$ is drawn to cut the sigmoid curve so that the area of the loop above p_0 is equal to the area of that below.

The link between Leiden and Belfast was strengthened when van der Waals's professor, P. L. Rijke, sent W. J. Janssen to learn from Andrews the techniques of studying fluids at high pressures. Janssen took equipment back to Leiden where he repeated for nitrous oxide Andrews's work on carbon dioxide. This he presented to the British Association meeting in 1876, when Andrews was President³⁴. From this modest beginning grew the great Dutch schools of experimental work on the equation of state at Leiden and Amsterdam.

Recognition came more slowly in Germany. As late as 1876 Clausius³⁵ made a further calculation of the covolume and, not knowing of van der Waals's result, made it eight times the volume of the molecules. van der Waals quickly corrected him³⁶. (In his review^{29,30} Maxwell had criticized van der Waals's result and gave, without derivation, an expression which is equivalent to sixteen times the volume!) In 1877 Wiedemann published a long abstract of the thesis in German³⁷, which was soon followed by Roth's translation² of the whole.

Once the value of the new equation became clear it was examined more carefully, with particular attention to the separation van der Waals had made between the two effects of molecules occupying space and of attracting each other, a separation which is at the root of what are now called mean-field and perturbation theories.

Mean-field Theories

When is the separation justified, and the calculation of U from equation (2) valid? The answer is for hard spheres with an attractive potential of so long a range that each interacts with many others. U can then be found correctly by averaging $u(r)$ over all configurations without regard to the microscopic structure of a dense fluid. Today this structure is described by a probability density $g(r)$, the ratio of the local density at distance r from a given molecule to the mean density. Hence

$$U = \frac{1}{2}(N^2/V) \int_0^\infty u(r)g(r)4\pi r^2 dr \quad (2')$$

and equation (2) follows if $g(r) = 0$ for $r < \sigma$, the diameter of a sphere, and $g(r) = 1$ for $r > \sigma$.

van der Waals knew that his results depended on such an assumption, for he wrote (ref. 3, pages 343, 361 and 435) "On the particles of a gas no forces act; on the particles within a liquid the forces neutralize each other", and again, that in a liquid "the energy will be the same for all positions which it [the particle] is free to assume". In modern terms, if $g(r) = 1$, the potential of average force is zero. Neither he³ nor Maxwell³⁸ was so clear that putting $g(r) = 1$ is justified only if $u(r)$ is weak but of long range. Rayleigh^{14,15} was more precise; there must be forces "whose range, though small in comparison with the dimensions of sensible bodies, is large in comparison with molecular distances. In the extreme case, the influence of the discontinuous distribution of the attractive centres disappears, and the problem may be treated by the methods of Laplace". Boltzmann was equally clear; in the first chapter of the second volume of his *Vorlesungen über Gastheorie*³⁹ he says the forces must be weak but of long range. When van der Waals saw this in proof he objected that he had never made this assumption, and that he doubted if it were true. Indeed, in his thesis³, he had used the fact that $2H$ is the first moment of K to show that the effective range of $u(r)$ is only 3 to 6 Å. Boltzmann replied³⁹ that "Nevertheless, I cannot obtain an exact foundation for his equation without this assumption". He was followed by Ornstein in 1908 (see refs 40 and 41, both of which refer to his thesis) in the first derivation of van der Waals's equation from Gibbs's statistical mechanics. The modern version of this work is that of Kac and others⁴²⁻⁴⁵ who have shown rigorously that the "van der Waals limit", as they call an equation of the form of equation (3), is implied by a potential

$$u(r) = -\alpha r^s \exp(-\gamma r) \quad (6)$$

where s = dimensionality, in the limit when the range γ^{-1} becomes infinite.

When interactions are short, $g(r) = 1$ is a drastic mean-field approximation widely used to discuss critical points, Curie points and order-disorder transitions of all kinds. Domb^{46,47} has pointed out that in 1889 Hopkinson discussed "the temperature at which the magnetism disappears which we may

but many who then took it up seemed unaware of the past, appropriately call the critical temperature". The name Curie point was introduced by Weiss and Onnes in 1910 after the former⁴⁸⁻⁵⁰ had, in 1906, proposed a mean-field treatment based on the reasoning of van der Waals; "On pourrait donner à NI le nom de champ intérieur pour marquer l'analogie avec la pression intérieure de van der Waals".

Mean-field approximations are surprisingly accurate except at critical points where they fail to describe the singularities of most physical properties as functions of T and v (or other extensive variable). The free energy from equation (5) is everywhere an analytic function and the behaviour it implies is most simply explored by expanding it in a Taylor series in $(T-T^c)$ and $(v-v^c)$. This was done by van der Waals^{51,52} in 1894 and in more detail by Verschaffelt⁵³⁻⁵⁴ in 1902. The failure of this simple Taylor expansion to describe the critical point was clear by the beginning of this century and in 1923 Verschaffelt⁵⁵ devised an expression for the pressure which had some of the required singularities, but interest was now waning and his work was not followed up. When critical phenomena again became an active field after 1950, one of the first attempts⁵⁶ to devise an acceptable form of the pressure was similar to that of Verschaffelt. Later work⁵⁷ has led to more satisfactory functions, the so-called scaling functions, the description of which is outside the scope of this article.

Perturbation Theories

If $u(r)$ is everywhere weak then $y = |u(r)|/kT$ is small and so a perturbation expansion in y is a natural way to calculate the phase integral. In the van der Waals limit y tends to zero and only the first term contributes to the thermodynamic properties. If, however, $u(r)$ is, more realistically, of a range comparable with σ and has a minimum of $-\epsilon$ then $y_{\max} = \epsilon/kT$ is of the order of unity at the critical point. It is now not obvious that an expansion will converge rapidly, but fortunately it does so. If the initial potential is that of a hard sphere, this can be "perturbed" into a realistic form by adding an attractive term⁵⁸ and softening the repulsion⁵⁹. Barker and Henderson and others^{60,61} have used such perturbation expansions to obtain a quantitatively successful theory of fluids. Their success depends on the fact that $g(r)$ in a dense fluid is determined chiefly by the geometry of packing of the hard cores, and so, to the first approximation, U can be found from equation (2') by putting $g(r)$ equal to $g_0(r)$, the distribution function for the hard spheres. This step is in direct descent from van der Waals's separation of the attractive and repulsive forces⁶².

Principle of Corresponding States

Substances to which equation (5) applies conform to a principle of corresponding states, for the three critical constants can be expressed in terms of R , a , and b . van der Waals⁶³ established this in 1880 and the principle at once became a useful tool in deciding how to liquefy the so-called permanent gases, for it enabled critical and Joule-Thomson inversion temperatures to be estimated⁶⁴. He always associated the principle with the two parameters a and b of his equation, but today we accept it as the consequence of a set of conformal intermolecular potentials; that is, those of a common geometric form with two scaling parameters σ and ϵ . Kamerlingh Onnes and others⁶⁵⁻⁶⁷ suggested that a condition of mechanical similarity lay behind the principle, but a clear statement^{68,69} of this condition came only in 1938.

Surface Tension

If the first Laplace constant K leads, by way of equation (2), to van der Waals's equation, what can be found from his second constant H ? Laplace supposed that a liquid has a sharp surface at which the density falls abruptly from that of

the liquid to the negligible density of the gas. This assumption was made later by Fowler⁷⁰ who thereby obtained

$$\gamma = 1/32 (N/V)^2 \int r^2 (du(r)/dr) g(r) dr \quad (7)$$

If again we put $g(r) = 1$ and integrate by parts then we obtain, with Laplace and van der Waals, $\gamma = \frac{1}{2}H$.

Laplace's derivation was criticized by Poisson for the assumption of a sharp interface, and Fowler's similarly by Kirkwood and Buff⁷¹ who obtained a more generally valid form of equation (7) in which $g(r_{ij})$ is a function of the positions in the interface of molecules i and j .

van der Waals came also to realize that this change of density with height must be taken into account, but his method of doing so was different^{51,52}. He assumed that the free energy contained terms proportional to the derivatives of the density ρ with height z and so, by arguments too long for summary, found

$$\gamma = -C \int \rho (d^2 \rho / dz^2) dz = C \int (d\rho / dz)^2 dz \quad (8)$$

The parameter C is proportional to the second moment of $u(r)$, not the first moment H , as for a surface of zero thickness. Maxwell had attempted to obtain expressions of this kind, but his work was criticized by Rayleigh⁷² who arrived independently at essentially the same result as van der Waals.

This approach was revived by Cahn and Hilliard⁷³ and, in the hands of Widom and others (refs. 74-76 and work by J. Yvon cited there), is now the principal tool for studying surface tension in the critical region where the thickness of the interface becomes long compared with the range of $u(r)$. The parameter C , the second moment of $u(r)$, becomes the second moment of the direct correlation function $c(r)$ in the modern version⁷⁴⁻⁷⁷. That the range of $u(r)$ and $c(r)$ is the same is the Percus-Yevick approximation.

Theory of Mixtures

From 1889 to 1912 van der Waals worked on the extension of his equation to mixtures^{5,78,79}. In principle this is simple; because a and b arise from binary interactions they become in a mixture of composition x

$$a_x = \sum_{i,j} x_i x_j a_{ij} \quad b_x = \sum_{i,j} x_i x_j b_{ij} \quad (9)$$

He thus found an equation which, by choice of the parameters a_{ij} and b_{ij} , could, even for a binary mixture, represent a great number of phase equilibria in systems with up to three fluid phases and three critical lines⁸⁰. His theory led to a big experimental programme at Leiden and elsewhere in which these complications were unravelled^{79,81}. After 1914, however, physicists forgot both theory and experiments, which had to be rediscovered by chemists and chemical engineers after 1945. In 1962 Leland recovered equation (9), in its modern guise with the inter-molecular parameters $(\epsilon_{ij}, \sigma_{ij}^3)$ for a_{ij} and (σ_{ij}^3) for b_{ij} , but it was a further six years before it was reidentified as the van der Waals approximation^{82,83}. This equation, together with closely related perturbation expansions⁸⁴⁻⁸⁷, are now the most satisfactory theory of fluid mixtures.

Legacy

In each of these fields the ideas of van der Waals and his school lie behind much of modern work, and it is worth asking why they were so neglected after 1914. First, perhaps, because there were then new fields to explore. The Dutch experimental school turned to low temperature physics, and theoreticians everywhere to quantum mechanics. Second, there was an impasse in the theory of fluids because of ignorance of the origin and form of $u(r)$, a difficulty which needed quantum mechanics for its solution. By 1930 the field was open again,

and often explored what turned out to be the blind alley of lattice theories. With hindsight, however, the reintroduction of the ideas of the van der Waals era can be plotted in more precise form. No exact priorities can be given but, in outline, the timetable runs as follows: Taylor expansions about critical points (1935, 1937 (refs 88 and 89)), the theory of the pair distribution function (1935 (ref. 90)), of surface tension (1937 (ref. 70)), 1948 (refs 74–76), 1949 (ref. 71), 1958 (ref. 73), of conformal potentials and corresponding states (1938–1939 (refs 68 and 69)), non-lattice theories of mixtures (1951 (ref. 84)), 1962 (ref. 82), 1968 (ref. 83), the direct correlation function (1953 (ref. 91)), and perturbation theories of pure fluids (1954 (ref. 58)), 1963 (refs 42–45), 1964 (ref. 59), 1967 (refs 60 and 61). Other ideas, not discussed here, have played at least as important a part in the current renaissance of liquid state physics, but in the papers cited above one can see the true legacy of van der Waals and his school.

I thank Dr H. J. Michels, Miss V. A. Milne and Professor J. G. Powles for their help.

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Cover Cartoon

The cover illustration of this issue of *Nature* depicts the British Association for the Advancement of Science as seen through the eyes of a *Punch* cartoonist in September 1865 when the association held its annual meeting in Birmingham. At that time, Sir Roderick I. Murchison, who is seen conjuring in the foreground of the cartoon, was President of the Geology Section. Presumably Professor T. H. Huxley and Dr Richard Owen are the two gentlemen embracing each other.

Identification this far is easy, but the names of the remaining

scientists pose problems. Who indeed are the geologists demonstrating, before their time, the subtleties of plate tectonics? And who is that brandishing what looks suspiciously like a red hot poker? The caption to the cartoon poses a problem as well. "The British Association illustrated by experimental conjuring (a box of tricks packed up in a large clothes-basket for doing the Stodare trick. Swords separate.)" Who or what was Stodare? The first reader to convince the Editor—by letter—that he can identify the scientists and also interpret the caption will receive a free subscription to *Nature* for a year.

Prebiotic Activation Processes

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A series of reactions involving urea, imidazole, and magnesium can result in the activation of nucleotides and amino acids to form polymers. Unlike any which have been suggested before, these reactions have parallels in contemporary biological mechanisms.

PREBIOTIC syntheses of many of the twenty naturally occurring amino acids and of the four standard ribonucleotides have been described^{1,2}. It seems natural, then, that we next ask how these compounds were induced to combine to form polypeptides and polynucleotides. Here we suggest a possible solution to a simplified but well defined version of this problem, the activation of a simple mixture of amino acids and nucleotides. The prebiotic soup, no doubt, contained many other compounds, some of which would have complicated the reaction scheme that we propose. We think, however, that a discussion of this oversimplified problem is a necessary introduction to any treatment of a more realistic prebiotic soup.

Two groups of prebiotic condensation reactions have previously been discussed extensively. In the first, monomers are heated together in the absence of water^{1,3}. In the second, unsaturated molecules such as cyanamide^{1,4} and cyanoacetylene⁵ are used as dehydrating agents in aqueous solution. Neither of these groups of reactions resembles contemporary biochemical pathways.

Here we describe a series of reactions that have much in common with the activation steps that precede protein and nucleic acid synthesis. Each reaction occurs in the solid state in plausible prebiotic conditions. Together they provide the basis for a unified scheme of amino acid and nucleotide activation. Urea, imidazole and Mg^{2+} are at present essential catalytic components of our reaction mixtures, but it is probable that other organic molecules could replace them.

Chemical Nature of the Primary Energy Reserve

The energy for most biological syntheses is provided by the hydrolysis of pyrophosphate bonds. ATP is the main energy source, but other compounds, for example GTP, are used occasionally. It seems unlikely that a highly specific synthesis of ATP or GTP occurred on the primitive Earth. Instead, we propose that the primary chemical reserve of free energy on the primitive Earth was a mixture of inorganic and organic polyphosphates.

This proposal is not new^{1,2}, but it is made more plausible by the discovery of a reaction in which urea, in the presence of NH_4^+ , catalyses the formation of inorganic and organic polyphosphates from inorganic phosphate at temperatures as low as 65° C⁶⁻⁸. The arguments that follow, however, do not depend on the way in which the polyphosphates, that we assume to have been present on the primitive Earth, were formed. They could have been made directly from an acidic phosphate in a volcano, or by some undiscovered catalytic or photochemical process, rather than by the urea-

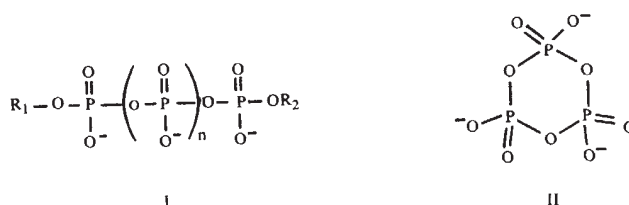


Fig. 1 I, $R_1 = R_2 = H$; $n = 0, 1, 2 \dots$ inorganic phosphate. $R_1 = \text{Nucleoside}$, $R_2 = H$; $n = 0, 1$ nucleoside di- and triphosphates. $R_1 = \text{Nucleoside}$, $R_2 = \text{nucleoside}$; $n = 0$ dinucleoside pyrophosphate. II, Inorganic trimetaphosphate.

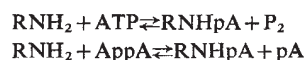
catalysed condensation. To make the discussion more concrete we shall nonetheless suppose that the polyphosphates available on the primitive Earth were of the types formed by heating mixtures containing urea, ammonium phosphate and metal ions in various proportions at moderate temperatures⁸ (Fig. 1).

Inorganic Catalysts

Pyrophosphates do not react readily with amino or carboxyl groups in aqueous solution. Thus it is hardly surprising that the coupling of the hydrolysis of linear polyphosphates and the formation of peptide bonds in aqueous solution is inefficient. Furthermore, we have found that very little reaction other than breakdown occurs if a dry mixture of glycine and the sodium salt of ATP is warmed.

Lowenstein has reported that acetate ion can be phosphorylated by ATP in aqueous solution, in the presence of divalent ions, to give acetyl phosphate⁹. The dismutation of ATP to ADP and higher polyphosphates in the presence of catalysts has also been reported¹⁰. This led us to examine the behaviour of amines including amino acids, when warmed gently with the magnesium salt of ATP in solution and in the solid state. In this way one of us (R. L.) discovered a novel and general series of solid-state reactions.

Amines, including ammonia, glycine and ethylenediamine, react with ATP or AppA in the dry state, if enough Mg^{2+} is present, to form phosphoramidates.



Imidazole reacts in a precisely parallel fashion to form ImpA. The yields in these reactions range from a few % in the case of imidazole to 40% in the case of ethylenediamine. We do not yet know how general this catalytic action of Mg^{2+} is, nor have we studied the phosphoramidates of the type $RNHPO_3H^-$ that are presumably formed along with the adenylic acid derivatives. Our results show, however, that, if the prebiotic soup were rich in Mg^{2+} , polyphosphates would have reacted with amino acids on evaporation to form derivatives of the type shown in Fig. 2.

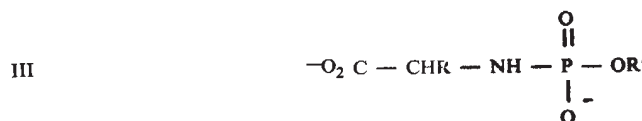


Fig. 2 $R' = \text{Nucleoside (a) or } R' = H \text{ (b)}$.

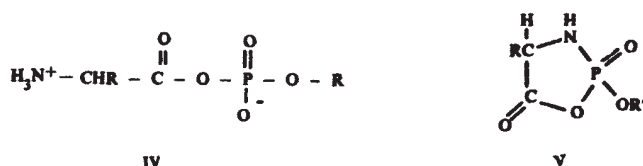


Fig. 3

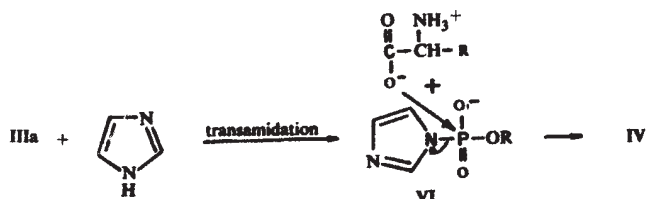


Fig. 4

There is another plausible prebiotic source of phosphoramidates of the special class IIIb, namely, the reaction between amino acids and inorganic trimetaphosphate in aqueous solution¹¹ or in the solid state (N. M. Chung, unpublished). This reaction, however, cannot be extended to the production of amidates or nucleotides.

Organic Catalysts

The phosphoramidates discussed in the previous section are activated at the phosphate group. The phenylalanine adduct of d-pApA has been reported to condense to short oligonucleotides on a poly (U) template¹². In our studies of template-directed synthesis we have found the imidazole derivative of pA condenses very efficiently on a poly (U) template to give di- and short oligonucleotides¹³. Thus the Mg²⁺-catalysed reactions of amino acids (or any other amine or imidazole) would provide nucleotide derivatives that can take part directly in prebiotic polynucleotide synthesis.

The problem of amino acid activation, however, remains to be resolved. Although the phosphoramidates of the amino acids are energy-rich compounds, they cannot react directly to form peptides, as the carboxyl groups are not activated. The following considerations, however, suggest that, in the presence of an appropriate catalyst, the N-phosphorylated amino acids might behave kinetically as though they were carboxyl-activated.

Phosphoramidates are exceedingly resistant to hydrolysis in alkaline solution, while the aminoacyl adenylates IV (R=adenosine) (Fig. 3) are extremely labile; the rates of direct hydrolysis probably differ by a factor of 10⁵ or more at room temperature and pH 8.5. On the other hand, the free energy content of the anhydride is not likely to exceed that of the phosphoramidate by more than 5 kcalorie. This suggests that at least 1/10% of the anhydride would be in equilibrium with the phosphoramidates at room temperature. Thus, if equilibrium were approached rapidly enough, a phosphoramidate of an amino acid would behave kinetically as an aminoacyladenylate. This is an interesting possibility because aminoacyladenylates are the intermediates in protein synthesis.

The phosphoramidate III could be converted to the anhydride IV by an intramolecular or by an intermolecular mechanism. Ring compounds such as V (Fig. 3) would be involved in the intramolecular route; they have been discussed a number of times in related contexts¹⁴. Here we consider the alternative intermolecular mechanism (Fig. 4), because it provides a simple explanation of some of our recent results.

If the phosphoramidate III (R=H, CH₃) is heated (65–100° C) alone no peptide is formed, but in the presence of excess imidazole di- and short oligopeptides are formed in

yields of up to 10%. A possible mechanism involves the formation of ImpA (VI) as an intermediate (Fig. 4).

We have confirmed that a higher yield of peptide is obtained when ImpA is heated with glycine in the same conditions, and that carboxylic acids like benzoic acid react with ImpA to give the anhydride of benzoic and adenylic acid. It is also interesting that the synthesis of the phosphoramidate IIIa (R=adenosine) and its subsequent condensation to short peptides can be achieved in a single operation if ATP, glycine, imidazole and Mg²⁺ are heated together.

If aminoacyladenylates (IV) (R=adenosine) are generated in this system they would react with free imidazole to form a certain amount of the corresponding aminoacylimidazolides VII (Fig. 5). Some of the peptide formed may be derived from this reactive intermediate rather than from the aminoacyladenylate.

Parallels with Biochemistry

The direct synthesis of peptides in the solid state has little in common with contemporary biochemical processes. But the intermediates which we believed to be formed in the solid state do resemble contemporary reactive intermediates. The aminoacyladenylates are the substrates of protein synthesis. Many phosphate kinases and related enzymes form intermediates in which histidine or lysine is attached to phosphate by an N–P bond^{15,16}.

We propose, therefore, that activated intermediates for prebiotic condensation reactions were formed in the solid state and subsequently reacted in the presence of small amounts of water to form oligonucleotides and oligopeptides. The solution reactions may have evolved into polynucleotide and polypeptide synthesis.

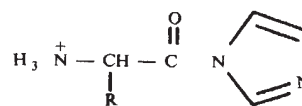
The aminoacylimidazolides and the aminoacyladenylates both react specifically with hydroxyl groups in preference to water (R. Saffhill and C. K. Biebricher, unpublished). It is thus likely that a proportion of the activated intermediates, instead of reacting directly to form peptides, would react with adenylic acid in aqueous solution to form the 2'(3')-ester. This reaction resembles the step in protein synthesis in which the amino acid is transferred from an aminoacyladenylate to the terminus of tRNA.

Our complete scheme for amino acid and nucleotide activation is summarized in Fig. 6. For simplicity and to emphasize the relation to biochemistry we have written all polyphosphates as derivatives of adenosine. This is not an essential part of our proposal.

Prebiotic Organic Catalysts

The scheme that we propose involves imidazole as a catalyst. It seems almost certain that many substituted imidazoles would behave in a similar way. Potentially prebiotic syntheses of such compounds are already well known. (a) Sugars react with ammonia in a variety of conditions to give imidazoles, particularly hydroxymethylimidazoles¹⁷. (b) The adduct of ammonia and cyanoacetylene is converted to imidazole by ultraviolet radiation¹⁸. (c) Sugars react with formamidine to give imidazoles. Imidazole glycerol phosphate, for example, is obtained from ribose 5'-phosphate and formamidine¹⁹. Imidazole glycerol phosphate is an intermediate in the biosynthesis of histidine²⁰.

Histidine is an imidazole derivative that plays a special



VII

Fig. 5

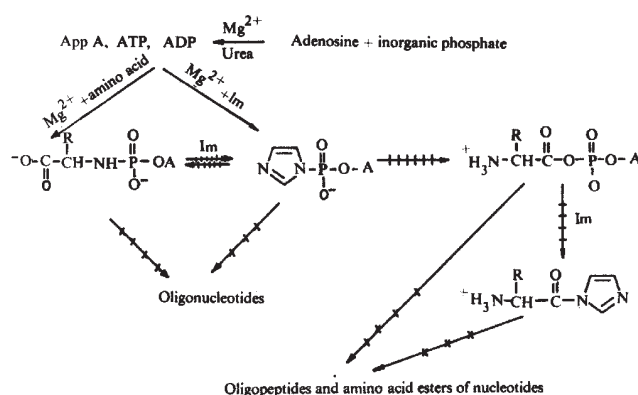


Fig. 6 A scheme for the prebiotic activation of nucleotides and amino acids. $\longrightarrow$, Solid state reaction; $\rightleftharpoons$, reaction which occurs either in the solid state or in solution; $\nrightarrow$, reaction which occurs in solution and imitates a step in a non-biochemical sequence.

role in contemporary biochemistry, as an amino acid present at the active site of a variety of enzymes. Unfortunately, no convincing prebiotic synthesis of this amino acid has been reported. If such a synthesis could be found, a detailed investigation of the catalytic properties of histidine and its peptides in this system would be justified.

No doubt, other organic catalysts exist that could replace the imidazoles; pyridines, for example. If we restrict our discussion to molecules related to those that are important in biochemistry, however, few possibilities are left for consideration. Of these, the thiols are the most interesting. Many enzymatic acylation reactions proceed through thioacyl intermediates, and thiophosphates are sufficiently activated to

take part in polynucleotide synthesis. Unfortunately, very little is known about the relevant chemistry, so evaluation of this alternative must await further work on the unit reactions involved.

This work was supported in part by grants from the US National Aeronautics and Space Administration and the US National Institutes of Health.

Received April 17, 1973.

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Effect of Actinomycin D on DNA Synthesis in Liver

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Studies with actinomycin D do not support an essential role for transcription concomitant with nuclear DNA replication in the liver.

THE inability of purified DNA polymerases to initiate the replication of intact double stranded DNA or single stranded circles of DNA has suggested that 3'-termini must be generated as part of the initiating event. A body of evidence is now accumulating that RNA, covalently linked to DNA, provides the 3'-terminus in a variety of systems including bacteria¹⁻³, phages⁴⁻⁷, extrachromosomal bacterial bodies^{8,9}, and eukaryotes¹⁰⁻¹³. The replication of ϕ X174 may be an exception as its DNA formation can be initiated in the absence of RNA precursors⁷ and is not blocked by rifampicin¹⁴. To

assess the role of RNA synthesis during the S period of liver nuclei *in vivo*, the effect of a high level of actinomycin D on DNA formation has been studied in regenerating rat liver after partial hepatectomy.

Female Sprague-Dawley-derived rats were freely given food and water at all times and partially hepatectomized when they weighed about 100 g. Partial hepatectomy was the removal of 70% of the liver and, unless otherwise indicated, injections were made in the tail vein.

DNA and RNA Synthesis

To study the effect of actinomycin on ongoing DNA replication, partially hepatectomized rats were prelabeled with ¹⁴C-thymidine and were then given 0.5 mg of the antibiotic. At various intervals thereafter, DNA synthesis was measured with ³H-thymidine (Fig. 1). The results, expressed as the ratios of the counts incorporated after and before treatment with the antibiotic, show that actinomycin caused a slow decline

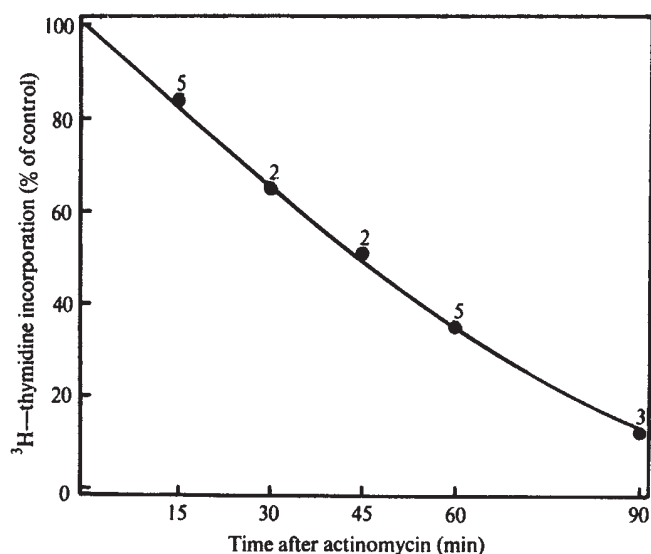


Fig. 1 Effect of actinomycin D on liver DNA synthesis *in vivo*. Partially hepatectomized rats were each given 0.1 μ Ci of 14 C-thymidine at 22 h after the operation. After 45 min, some of the animals received 1 ml of 0.15 M NaCl, the remainder 1 ml of a solution of actinomycin D (0.5 mg ml $^{-1}$). At 15 to 60 min after the second injection, each rat was given 5 μ Ci of 3 H-thymidine and liver samples were taken 15 min later. The values shown were calculated from the ratios of 3 H-DNA/ 14 C-DNA and are the averages of the individual results with two to five animals, as indicated. The average ratio for six control animals that had been given saline solution instead of actinomycin (6.2 ± 0.4) was taken to be 100%.

in 3 H-thymidine uptake and that even after 45 min the reduction was only 50%.

RNA synthesis in the regenerating liver was almost completely abolished by 0.5 mg of actinomycin. Thus, the specific activities of acid-washed nuclei from rats labelled with 3 H-orotate (50 μ Ci) for 5 min beginning at 15 min after the injection of a saline solution of actinomycin were 144,000 and 1,680 c.p.m. per g of wet liver, respectively. All the radioactivity was made acid-soluble by RNase and by NaOH. Almost identical results were obtained when the 3 H-orotate was given with actinomycin in a single solution, indicating that RNA synthesis was stopped immediately.

The inhibition of RNA labelling by actinomycin was not due to a block in the conversion of orotate to UTP. The specific activities of regenerating liver UTP 15 at 5 min after the injection of 3 H-orotate (10 μ Ci) were 1.9×10^5 and 1.7×10^5 c.p.m. μ mol $^{-1}$ in control animals and in animals that had been given 0.5 mg of actinomycin 15 min earlier, respectively.

Autoradiographic Analysis

Partially hepatectomized rats, prelabelled with an amount of 14 C-thymidine (0.05 μ Ci) that would not interfere with

autoradiography, were given actinomycin and, after various intervals, 100 μ Ci of 3 H-thymidine (Table 1). As can be seen from the ratios of 14 C-thymidine uptake/% of 3 H-labelled nuclei, most of all replicating nuclei continued to make DNA even after 60 min of exposure to the antibiotic. The ability of the replicating nuclei to incorporate 3 H-thymidine was lowered by the antibiotic so that the average number of grains per labelled nucleus was gradually reduced. The autoradiographic results are not consistent with the possibility that the thymidine incorporated by the rats treated with actinomycin was for the repair of DNA rather than for replication.

Rates of Elongation of DNA

The reduced uptake of thymidine in the rats treated with actinomycin was caused by a decrease in the rate of growth of DNA strands (Table 2). Thus, the incorporation of 3 H-thymidine into DNA and the average rate of chain elongation *in vivo* were both depressed by 90% at 1.5 h after injection of the antibiotic.

Table 2 Effect of Actinomycin on Rate of Elongation of DNA *in vivo*

Actinomycin	DNA formed (% of control)	Rate of chain elongation (nucleotide min $^{-1}$)
—	100	1,270 (1,130, 1,410)
+	13	127 (88, 114, 178)

Partially hepatectomized rats, prelabelled with 14 C-thymidine (0.1 μ Ci), were given saline solution or 0.5 mg of actinomycin at 20 h after the operation. After 90 min, 200 μ Ci of 3 H-thymidine was injected into the mesenteric vein, liver samples were removed 1 min later, and nuclear DNA was isolated. "DNA formed" was estimated from the ratios of 3 H/ 14 C counts. The DNA was hydrolysed in such a way that internal molecules were released as deoxy-nucleotides, molecules at the 3'-OH ends of strands, as deoxy-nucleosides 16 , and average rates of chain elongation were calculated from the ratios of thymidine/TMP that were separated chromatographically on 'Dowex' 1 (ref. 17).

With regenerating liver nuclei that are isolated and assayed under the conditions described 17,18 , only nuclei that were replicating DNA *in vivo* were able to incorporate 3 H-TTP *in vitro*; initiation of DNA synthesis does not occur, and most or all of the DNA strands that were already growing *in vivo* elongate. The properties of nuclei isolated from animals treated with actinomycin were indistinguishable from those of control nuclei with regard to 3 H-TTP incorporation, deoxy-nucleotide and ATP requirements for DNA synthesis, and the average rate of growth of DNA chains (Table 3). To support the validity of the rate measurements *in vitro*, Table 3 shows the results obtained when actinomycin D was added to the isolated nuclei. The antibiotic caused identical inhibition of 3 H-TTP incorporation and of the rate of strand elongation. The results *in vivo* as well as with the isolated nuclei strongly suggest that actinomycin did not reduce the number of DNA strands that were already growing before treatment.

Table 1 Effect of Actinomycin D on Labelling of Individual Nuclei with 3 H-Thymidine

Time with actinomycin (min)	DNA formed		3 H/ 14 C	% Labelled nuclei	Labelled nuclei	
	C.p.m. 14 C mg $^{-1}$ DNA	C.p.m. 3 H mg $^{-1}$ DNA			Grains/nucleus	C.p.m. 14 C mg $^{-1}$ DNA / % labelled nuclei
*	1,060	2.5×10^5	236	29	17	37
15	940	1.9×10^5	202	25	13	38
30	1,260	2.0×10^5	159	30	12	42
45	1,710	1.8×10^5	105	38	8.5	45
60	1,320	1.0×10^5	76	32	6	41

Partially hepatectomized rats were given 0.05 μ Ci of 14 C-thymidine at 22 h after the operation. After 45 min, each animal was given 0.5 mg of actinomycin and, 15 to 60 min later, 100 μ Ci of 3 H-thymidine. Liver samples, taken 60 min after the last injection, were used for autoradiography and for the estimation of the specific activities of the 14 C and 3 H-labelled DNAs. Labelled nuclei were scored in parenchymal cells only. Each value for the "grains per nucleus" is the average of the results with 200 to 400 nuclei and background was taken to be 1 grain per nucleus.

* The rat received saline solution in place of actinomycin.

Table 3 ^3H -TTP Incorporation by Isolated Liver Nuclei from Rats Treated with Actinomycin and Rate of Elongation of DNA Strands *in vitro*

Actino- mycin	Additions (+) or omissions (-)	DNA formed (c.p.m. mg^{-1} DNA)		Rate of chain elongation (nucleotides min^{-1})
		<i>in vivo</i>	<i>in vitro</i>	
-	None	2,460	57,100	194
	-dATP, dCTP, dGTP		1,380	
	-ATP		7,960	
	+ actinomycin (3 $\mu\text{g ml}^{-1}$)		35,200	126
	(10 $\mu\text{g ml}^{-1}$)		20,700	65
+	None	2,090	42,600	223
	-dATP, dCTP, dGTP		1,030	
	-ATP		7,550	

Partially hepatectomized rats were labelled with 0.1 μCi of ^{14}C -thymidine at 22 h after the operation and, 45 min later, some of the animals received a saline solution and the others received 0.5 mg of actinomycin D. At 60 min after the second injection, liver nuclei were isolated and incubated for 5 min or 1 min ("rate of chain elongation") in reaction mixtures containing ^3H -TTP¹⁷. Rates of DNA strand growth were estimated as in Table 2. Each value is the average of the results of two experiments.

Density Gradient Analyses

To explore the possibility that the RNA needed to initiate liver DNA synthesis was formed even in animals treated with actinomycin, rats that had been given antibiotic (0.5 mg) 15 min earlier were labelled for 5 min with a mixture of ^{14}C -thymidine (1 μCi) and ^3H -orotate (250 μCi). Nuclear DNA was isolated, denatured by heating (80° C for 10 min) in 12% formaldehyde, and centrifuged in an equilibrium gradient of neutral CsCl . No labelled RNA was detected in the area of the ^{14}C -DNA. The same results were obtained with liver DNA from partially hepatectomized animals not given actinomycin.

Different results were obtained when 90% formamide was used for denaturation (37° C for 30 min). In this case, DNA from partially hepatectomized rats not treated with actinomycin, showed a sharp ^3H peak which was slightly more dense than most of the DNA and which was made completely acid-soluble with RNase and NaOH. Identical results were obtained, however, with animals with intact livers even though the level of DNA synthesis was less than 5% of that after partial hepatectomy. It would seem that formamide, unlike formaldehyde, did not denature all RNA-DNA hybrid molecules. Incomplete denaturation rather than covalent linkages may explain some of the positive results that have been reported with eukaryotic cells.

In the conditions of the experiment using formaldehyde, the specific activity of UTP was found to be 5×10^6 c.p.m. μmol^{-1} and, taking a value of 4 nmol mg^{-1} of liver DNA for the pool size of TTP¹⁹, the specific activity of the deoxynucleotide was 0.5×10^6 c.p.m. μmol^{-1} . As the peak fraction of ^{14}C -DNA in the gradient contained 200 c.p.m., labelled RNA should have been detectable in the DNA area if the ratio of radioactive nucleotides in RNA and DNA were no lower than 1:200. A value of 1:2 or 1:4 has been found for very nascent Okazaki fragments in *Escherichia coli*.

Evidence for RNA covalently linked to liver DNA was also sought by comparing the densities of nascent DNA (0.5 min labelling period with ^3H -thymidine) and bulk DNA from partially hepatectomized rats that had not been treated with actinomycin. Equilibrium centrifugation in neutral gradients of CsCl showed that the nascent DNA had exactly the same density as most of the DNA. Identical results were obtained with DNA that was extensively sheared by sonic oscillation (about 900 nucleotides)¹⁷ before centrifugation.

TTP and Deoxynucleotide Synthesis

Actinomycin (0.5 mg) depressed by about 30% the ability of the liver to convert ^3H -thymidine into ^3H -TTP at 90 min

after the injection. The antibiotic caused an even greater reduction in the synthesis of cytosine deoxynucleotides from cytidine. Thus, at 90 min after injection of partially hepatectomized rats with a saline solution or 0.5 mg of actinomycin D, the radioactivity (c.p.m. mg^{-1} DNA) in liver cytosine deoxynucleotides was 6,080 (range 5,500 to 6,650; three rats) and 2,040 (range 1,390 to 3,300; five rats), respectively, as measured after labelling for 5 min with 250 μCi of ^3H -cytidine²⁰. Entry of the ^3H -cytidine into the liver was not affected by the antibiotic as the total acid-soluble counts were the same in both groups of animals. The reduced availability of labelled and unlabelled substrates for DNA synthesis may account for the decrease in thymidine uptake in the rats treated with actinomycin.

No Essential Role

The results with actinomycin do not support an essential role for transcription concomitant with nuclear DNA replication in liver. A level of the antibiotic that almost completely stops RNA synthesis causes only a slow decline in DNA formation. The decline seems to be due to a reduction in the rate of elongation of DNA chains and not to a loss in the ability to start new chains. Finally, no evidence could be found for the existence in regenerating liver of RNA covalently linked to nascent DNA.

Nevertheless, the possibility cannot be ruled out that RNA molecules are required for the initiation of hepatic DNA synthesis. If this is so, however, it must follow that the RNA is made by a unique polymerase that is highly resistant to actinomycin D or that all the initiating RNAs are completed before or early in the S period or that the initiating RNA, unlike that of bacteria³, is needed only very infrequently during the replicative process. The last possibility is unlikely, for even after 45 min of exposure to actinomycin, about 10% of the S period, the reduction in thymidine uptake is only 50%.

Received April 16, 1973.

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LETTERS TO NATURE

PHYSICAL SCIENCES

New Method for Direct Determination of UT1

ALTHOUGH Atomic Time is now available (for example as co-ordinated time, UTC) to a precision far in excess of that to which any astronomical time scale can be determined, astronomers, navigators and surveyors, concerned with their position on the rotating Earth, still require a knowledge of Universal Time, UT1. Universal Time is obtained by calculation from observed sidereal time, usually measured by timing the passage of stars across the meridian with a transit circle, or, more recently, with a photographic zenith tube. This measure of Universal Time, UTO, contains irregularities due to both polar motion, sometimes known as Chandler Wobble, and variations in the rate of rotation of the Earth, and its value at any instant depends upon the position of the observer on the Earth.

Of much more general use is UT1, which is the same at all locations and provides a measure of the rotation of the Earth about a pole of mean position. The correction that must be applied for polar motion to derive UT1 from UTO relies upon measurements of the instantaneous position of the pole, obtainable only by combining observations of the variations of latitude made by several collaborating observatories situated at widely spaced longitudes.

The method described here, based on observations of radio sources with an east-west interferometer, provides a determination of UT1 that is independent both of the proper motion of stars and of the instantaneous position of the pole and has the added advantage over all optical methods of being largely independent of weather conditions. Some preliminary results are given, derived from recent measurements with the 5-km telescope at Cambridge¹.

The technique for measuring the positions of radio sources with east-west interferometers has been developed to a high precision, enabling right ascensions and declinations to be independently determined²⁻⁴. It will be sufficient here to outline the method of measurement of right ascension and to show how this may be applied to provide a determination of UT1.

The phase difference Φ between signals at a wavelength λ arriving at two aerials of an east-west interferometer of spacing d is given by

$$\Phi = 2\pi (d \sin H \cos \delta) / \lambda$$

where δ is the declination and H the hour angle of the source at any instant. If the measured phase Φ is compared with the value Φ_0 , calculated from an assumed hour angle ($H + \Delta H$) for the source, then

$$\Phi - \Phi_0 = -2\pi (d \Delta H \cos H \cos \delta) / \lambda$$

From 12 h observations made with H from -6 h to $+6$ h, the measurement of the component of $\Phi - \Phi_0$ varying as $\cos H$ thus provides a value for ΔH . With the 5-km telescope, an on-line computer plots the results of the 12 h observations on a graph plotter, derives the best fit curve as shown in Fig 1, and prints out the corresponding value of ΔH .

This difference, ΔH , between the measured and assumed values is usually interpreted so as to provide a correction to the assumed right ascension of the source, having taken due account of all aspects of timing. But when the right ascension

of a source has been determined, subsequent observations may be used instead to give measurements of time, provided that the overall phase of the instrument and atmosphere remains sufficiently constant during the 12 h period of observation⁵.

The cabling and receiver systems of the 5-km telescope have been designed to minimize phase variations arising within the instrument, and variations arising from external causes are, in general, dominant. As hour angle, and hence time, are determined from measurements of the relative phase of signals arriving at two aerials, only differences in the two ray paths are important. These differences arise from the curvature of the atmosphere and ionosphere and also from dissimilar heights of aerials above the geoid. Corrections for these effects are small⁴ and do not vary significantly with barometric pressure. Irregularities of water vapour content in the troposphere, however, produce phase variations^{6,7} for which no correction is possible and which limit the overall accuracy attainable. The irregularities are most pronounced during the daytime in summer but their occurrence is easy to recognize, as shown in Fig. 1b, and observations affected may then be given lower weight.

If the assumed longitude of the radio telescope is in error, there will naturally be a corresponding timing error. Somewhat surprisingly this error is, to first order, independent of polar motion, as may be seen from the following argument. The instantaneous position of the pole is conventionally measured by angles from the mean position, denoting small rotations about axes through the centre of the Earth of, x , towards Greenwich, and y , at right angles to x , towards the west. Departure of the pole from its mean position in general gives rise to: (i) a variation of latitude, which has no effect on the phase difference measured with an east-west interferometer; and (ii) a change in the direction of the local meridian and hence a variation of longitude. Thus for an interferometer situated at latitude ϕ and longitude Λ west, the departure of longitude from the mean position by $\Delta\Lambda$, in the sense of increasing the longitude westwards, is given by

$$\Delta\Lambda = (x \sin \Lambda - y \cos \Lambda) \tan \phi$$

This is the expression used to derive the correction relating UT1 and UTO. Finally, the displacement of the pole causes: (iii) a variation of the astronomical azimuth of an interferometer

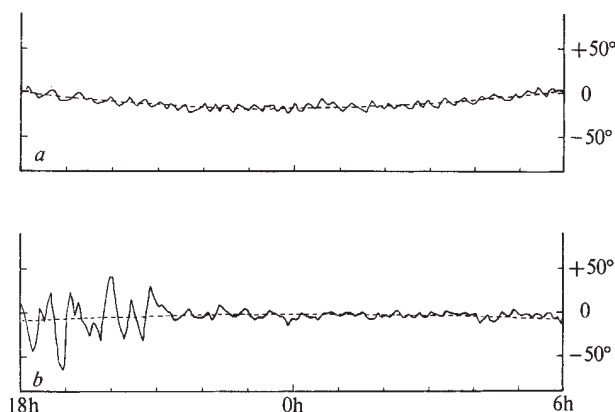


Fig. 1 The variation of $\Phi - \Phi_0$ with hour angle for 3C345. ---, Computer-fitted curve; a, difference in hour angle, ΔH , is $+12$ ms; b, $\Delta H = -6.5$ ms and also shows the effect of severe tropospheric irregularities.

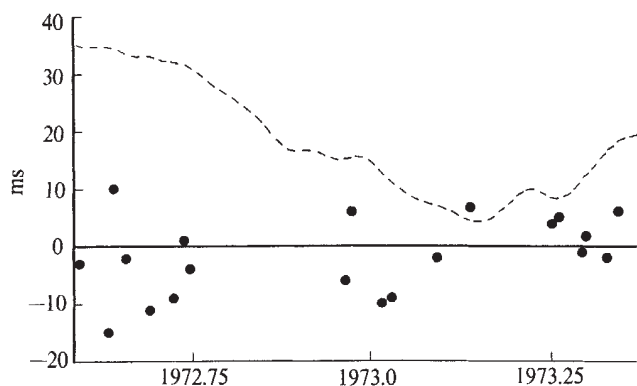


Fig. 2 ●, Differences between the measured and BIH values of (UT1-UTC), indicating the scatter in the radio measurements of UT1; ---, variation of (UT1-UTO), calculated for Cambridge.

fixed on the surface of the Earth. The change in azimuth ΔA , in the sense of decreasing the azimuth measured positive from north through east, is given by

$$\Delta A = (x \sin \Lambda - y \cos \Lambda) \sec \phi$$

This incremental change in azimuth may be resolved into two vector components, one parallel to the polar axis and another in a plane perpendicular to the polar axis. The component along the polar axis has no influence upon hour angle, but the other component, of magnitude $\Delta A \sin \phi$, is equivalent to an incremental change in longitude. But $\Delta A \sin \phi = \Delta \Lambda$ and so when the combined effect of the instantaneous position of the pole is considered, producing variations of both longitude and azimuth, the net result is zero and the effective longitude of the interferometer is independent of polar motion.

To investigate the use of the 5-km telescope to derive UT1, the radio source 3C345 was observed at intervals between August 1972 and May 1973. The longitude of the telescope was determined from measurements of β Persei, thus enabling the right ascension to be established⁴ relative to the FK4 fundamental catalogue⁷. The basic timing relies upon a quartz crystal clock, regulated to mean sidereal time. As the instrument is primarily used for aerial synthesis, for which it is frequently necessary to combine observations from one day to the next with the correct phase relationship, the clock is adjusted to follow UT1 closely. This is normally achieved by correcting for clock error, determined daily from the 60 kHz transmission of UTC from MSF Rugby, and by extrapolation of provisional values of UT1-UTC received weekly from the Bureau Internationale de l'Heure (BIH). The present observations have been analysed instead to give UT1-UTC independently of the data from the BIH.

The results are shown in Fig. 2, in which differences are plotted between the measured values of UT1-UTC and the corresponding values published by the BIH, thus giving a direct comparison of the two determinations of UT1 over the period. In addition, calculated values of UT1-UTO for Cambridge are indicated for comparison. There is excellent agreement between the measured and BIH values of UT1, the probable error of a single determination being ± 4.6 ms, which includes the error in the BIH value. This compares favourably with the external probable error of measurement of ± 18 ms from a single night's observations using a meridian circle with impersonal micrometer, and ± 12 ms when photoelectric registration is employed⁸. The corresponding value for the photographic zenith tube is ± 4 ms (ref. 8).

The early observations were made in daytime in summer and in consequence have larger errors than if they had been made at night with some other source. It should therefore be possible to achieve improved accuracy with the present instrument. Even more accurate measures of the rotation period of the Earth may be forthcoming from very long baseline interferometry⁹ and

from satellite and lunar laser ranging¹⁰, but the technique used by the 5-km telescope provides a self contained and fully automated measurement of UT1 that has the advantage of being available immediately at the end of a 12 h observing period.

I thank the staff of the Mullard Radio Astronomy Observatory for assistance and Professor Sir Martin Ryle for advice.

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Received June 18, 1973.

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Are the Jovian Planets "Failed" Stars?

THERE are three distinct types of object in the Solar System—the Sun is a star; the terrestrial planets (Mercury, Venus, Earth/Moon, Mars) are solid bodies; and the Jovian planets (Jupiter, Saturn, Uranus, Neptune) are cold gaseous bodies. The nature of Pluto is not established and Pluto will be discounted for my purposes here. It is surely no accident that the solid members of the planetary system lie nearer the Sun than the gaseous members. The most distant terrestrial planet (Mars) is 1.52 AU from the Sun whereas the nearest Jovian planet (Jupiter) is 5.20 AU from the Sun (1 AU = 1.496×10^8 km). As yet no agreed view of planetary formation exists. Most theories involve a single process for all the planets (see, for example, ref. 1). I suggest that the division of the planetary bodies into solid and gaseous types may imply different formation mechanisms. In particular I suggest that the terrestrial planets formed in a high density shell surrounding the proto-Sun and that the Jovian planets are the remnants of other attempts to form stars contemporaneously with the Sun.

Our numerical treatment² of collapse under gravity for spherically symmetrical gas clouds showed that collapse under gravity takes a characteristic form, which has also been found by others³⁻⁵.

Gravitational collapse takes place on a time scale determined by $\rho^{-1/2}$, where ρ is the mean initial density, in such a manner that the density increases slowly at first, but proceeds rapidly in the last 10% of the collapse time; increases of density of, say, fifteen orders of magnitude taking place in the last few per cent of the collapse time. This form of collapse means that for the collapse of two clouds differing in density by a factor of 9, while having collapse times differing only by a factor of 3, the higher density cloud will have completed its collapse to high densities before the lower density cloud will have been sufficiently compressed to increase its density by one order of magnitude. The implication of such a characteristic form of collapse is that, provided collapse can be maintained, a gas cloud, which has a monotonically decreasing density distribution from centre to boundary, will collapse such that the cloud may be considered in two parts—a rapidly collapsing small core of high density surrounded by a slowly collapsing low density

Table 1 Densities of Objects in the Solar System

Gaseous objects		Solid objects		Parent planet	Planetary satellites	
Object	Density *	Object	Density *		Object	Density *
Sun	1.409	Mercury	5.4	Earth	Moon	3.34
Jupiter	1.334	Venus	5.1	Jupiter	Io	5.0
Saturn	0.684	Earth	5.52		Europa	4.9
Uranus	1.60	Mars	3.97		Ganymede	3.0
Neptune	2.25			Saturn	Callisto	2.3
					Mimas	0.5
					Enceladus	0.8

* g cm⁻³.

envelope. It is the shell joining the high density core to the low density envelope which is of interest in the context of the formation of the terrestrial planets. I have drawn attention elsewhere^{4,7} to the effects of the density variation, by a factor of at least 10^{15} , and temperature variation, by at least a factor of 10^2 , within the shell linking core and envelope, on the rates of chemical reactions. At the highest densities and temperatures in the inner parts of the shell, chemical processes will take place very rapidly, producing many molecular species. Aggregation of molecular species into particulate matter would also be expected. Conditions are therefore appropriate for the formation of solid planets if the nature of the protostellar body is such that dispersal of the particulate matter does not take place soon after its formation, that is, conditions for proceeding to aggregation into planetary bodies may only be found for the less massive protostellar objects.

Any object which had collapsed to a high density condition would be surrounded by a shell in which extensive chemical evolution had taken place and particulate matter formed. One would expect that were the Jovian planets of this type some evidence of such chemical evolution would exist. In the case of Jupiter and Saturn circumstantial evidence in favour of the hypothesis does exist. Table 1 shows the densities of some Solar System objects⁸. All the solid planets have a mean density in excess of 3 g cm^{-3} whereas the gaseous planets have a mean density of $<2 \text{ g cm}^{-3}$ (with the exception of Neptune). One might conclude that the three inner satellites of Jupiter—Io, Europa and Ganymede—could be regarded as solid bodies. Saturn is surrounded by its rings of particulate matter and its nearest satellites, Mimas and Enceladus, are of very low density, comparable with the density of Saturn itself. The comparison of solar mean density with that of the Jovian planets is striking. The evidence, such as it is, does not conflict with the hypothesis that the Jovian planets may be remnants of other attempts to form stars.

If, however, "failure" to form a star can occur, one can legitimately ask why is it possible for a star to form in some situations and not in others, more particularly as it is clear that if the Jovian planets are "failed" stars, the collapse has proceeded almost to the stellar state? What could prevent ultimate evolution to the stellar state? Clearly it is not proximity to the Sun, for some binary stars have separations of $<0.05 \text{ AU}$. Some other force or forces must be at work and may exist in the dynamics of the star formation process.

The models of star formation based on gas clouds idealized to have spherical symmetry are unrealistic. As the gas clouds are observed to be irregular in shape and rotating, collapse under gravity will probably lead to flattened structures. As was originally demonstrated by Lin *et al.*⁹, a non-rotating spheroid will flatten during collapse. Rotation will enhance the tendency to flatten. But rotation is also seen as an inhibitor of collapse. As centrifugal forces amplify as l^{-2} whereas gravity amplifies as l^{-3} during collapse, where l is a characteristic linear dimension, it is clear that even if gravity dominates centrifugal forces originally, the greater amplification of centrifugal forces will cause them eventually

to dominate gravity. The conditions prevailing in interstellar gas clouds suggest that centrifugal forces should halt collapse soon after it starts. Much effort has been expended on mechanisms for removal of rotation of gas clouds, and the rotational problem is seen as one of the greatest difficulties encountered by any theory of star formation through collapse.

Numerical studies which we are carrying out on the collapse of gas clouds in two and three dimensions have indicated that such problems may be less severe than supposed. To make the problem specific, I shall consider the free-fall collapse of a uniform spheroid under gravity. Such a cloud maintains its uniform density and spheroidal shape during collapse, becoming progressively more and more flattened. The introduction of rotation about the minor axis of the spheroid does indeed reduce the collapse rate and may even cause equatorial expansion, but it does not prevent flattening. Indeed excess rotation does not cause the cloud to break up in these conditions, but merely generates an expanding ever thinning spheroid. Clearly other forces are necessary to cause breakup.

The extension of the work to cover the case of collapse of rotating gas clouds under gravity when pressure cannot be neglected is not yet complete. It is, however, clear that the halting of collapse by centrifugal forces acts progressively inwards as collapse proceeds. The region where infall can proceed for longest is that lying close to the axis of rotation. It follows that any accumulation of material at the centre of the cloud, through infall of material originally close to the minor axis, will have low angular momentum. If, however, the angular momentum of the cloud is large initially the centrifugal forces may effectively disperse the infalling material. Here then is a circumstance which could act at a late stage in the evolution of a gas cloud to the stellar state and inhibit actual attainment of that state. The numerical studies also suggest that details of the physical state may influence the dynamics of collapse in a sensitive way. Collapse once initiated may be frustrated by the interplay of many different factors and the occurrence of high density may not of itself ensure ultimate star formation.

The numerical studies in which we are at present engaged demonstrate very clearly that models of star formation which assume spherical symmetry are misleading. A spherically symmetric model shows no tendency to flatten. Any small departure from exact spherical symmetry will lead to flattening. Therefore there is no problem in producing flattened systems through collapse under gravity. Rotation, far from being a difficulty, may be necessary to produce a braking of the collapse for the bulk of the gas cloud, so preventing its eventual collapse onto any protostellar objects at its centre. The limitation of rate of accumulation of mass, through the imbalance of inflow near the axis of rotation of a cloud and outflow caused by centrifugal forces perpendicular to the axis of rotation, is clearly a factor controlling the growth or dispersal of accumulations of mass. Rotation may therefore be an essential component of the process of star formation, acting as a restraint on gravitation. As other forces are also acting on a collapsing cloud, the interaction is likely to be complex. It is quite clear, however,

that reliance on ideas derived from consideration of a spherically symmetrical model will lead to misconceptions in any attempt to understand the formation of stars through collapse under gravity.

The collapsing region out of which the Sun eventually formed will have contained density fluctuations which will have amplified during the collapse. Conditions of rotation and physical state will, however, have been such that only the density fluctuation now observed as the Sun could evolve to the stellar state; the other density fluctuations must have become inhibited and are now observed as the Jovian planets. Solar System objects such as asteroids and comets were then forced to develop within the constraints imposed by the forming Sun and its solid satellites—the terrestrial planets—and the attempted star formation by the Jovian planets.

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Received June 25, 1973.

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Rotation of the Earth during the 1972 Solar Event

Gribbin and Plagemann¹ have used the UT observations of the US Naval Observatory (USNO) as evidence of "a discontinuous change in the length of the day and a change in the rate of change of the length of the day" following a great solar storm in which the flare activity reached a maximum on August 4, 1972. They claim that variations in the UT observations of the USNO, soon afterwards, have confirmed their own prediction that such a storm should influence the rate of rotation of the Earth. Gribbin also asserts² that there was a 10 ms jump in

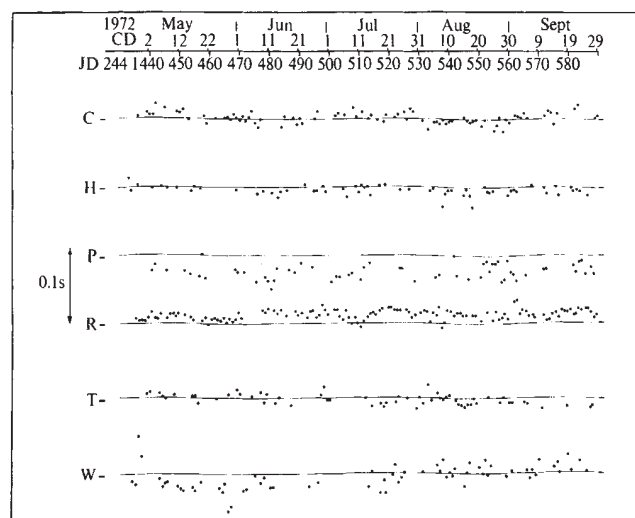


Fig. 1 UT2-AT, May to September 1972. C, Calgary photographic zenith tube (PZT); H, Herstmonceux PZT; P, Paris Astrolabe; R, Richmond PZT; T, Tokyo PZT; W, Washington PZT; —, Def. UT2-AT.

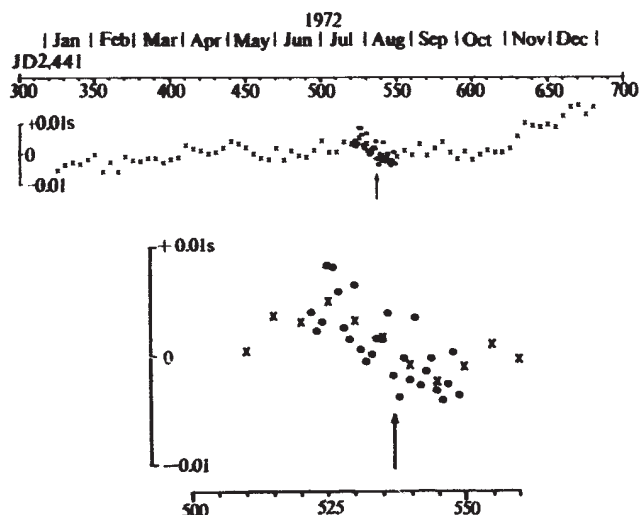


Fig. 2 UT2-AT, BIH raw values, determined from all data and corrected for drift of -3.2 ms d^{-1} . x, 5-d values; •, daily values.

AT-UT2 between August 7 and 8. The evidence is presented in the form of graphs of AT-UT2. (UT2 values are the UT observations corrected for observed polar motion and for a seasonal variation in the rate of rotation of the Earth, using the Bureau Internationale de l'Heure (BIH) adopted corrections.) The precise significance of the graphs is difficult to assess because of the irregular indexing of the axes. No mention is made of the instrument or instruments on which the data are based.

The astronomical observations of a single instrument can be systematically influenced by many factors, for example, errors in the adopted star places and instrumental or meteorological effects which can introduce an annual term in the results and random and unpredictable variations causing either a temporary wandering or an enhanced scatter. Uncorroborated results of any instrument over a brief period should therefore be treated with great caution, particularly where results of a worldwide network of instruments are available. The UT observations of many individual observatories are communicated weekly to the BIH where they are collated and analysed and corrected for systematic errors, and combined to establish a scale of Definitive UT (Def. UT).

In Fig. 1 we show the UT2 observations for individual nights during May to September 1972 of six instruments of maximum weight in the BIH scale of assessment. The plotted points represent the observed values of UT2-AT which, for convenience, have been corrected by application of a linear term $a+bt$ where $a=10.3984 \text{ s}$, $b=0.0032 \text{ s}$ and t is the number of days elapsed since JD 2441429.5. During this period the run of the observations is, in general, linear. Any change in the trend of the observations would indicate a change in the rate of change of the length of the day. Any discontinuity a sudden change in the length of the day. The dispersion in the observations is normal and the graphs illustrate how difficult it is to discern a significant change by the examination of the results of one instrument or, indeed, of the combined results of several instruments, over a few months.

From the BIH Annual Report for 1972, 5-d raw values of UT2-AT can be obtained, determined from all available data. In Fig. 2 we show these raw values corrected for a drift of -3.2 ms d^{-1} . For the period July 24 to August 20, 1972, daily raw values of UT2-AT have been supplied privately by the BIH. An enlargement of this section of the diagram is shown immediately below the plot for the whole year. August 7-8, 1972, is marked with an arrow.

The data of Fig. 2 demonstrate that small short-term variations frequently occur in the observed rate of rotation of the Earth, but that there was no significant change in the length of

We thank the staff of the RGO for assistance and H. M. Smith for discussions. The Paris Observatory, the Seismology Division of the Department of EMR, Ottawa, and the US Naval Observatory, Washington, provided unpublished data; the BIH and Tokyo data come from published circulars.

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Received May 22; revised July 2, 1973.

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THE earthquake occurred on December 23, 1972, at 0630 GCT. It had a magnitude $M_b = 5.7$ ($M_s = 6.1$) and a superficial focus of about 5 km. The epicentral region, which did not exceed in diameter more than a few kilometres, comprised the central part of the city of Managua where, out of a population of

Apart from falls and slides of debris along steep stream banks and road cuts, the earthquake of 1972 did not cause any foundation failures or liquefaction. In general, the foundation materials in Managua are intrinsically competent and they cannot be held responsible for the damage that the city suffered. Damage was local and varied erratically over short distances. This was chiefly because, with few exceptions where design

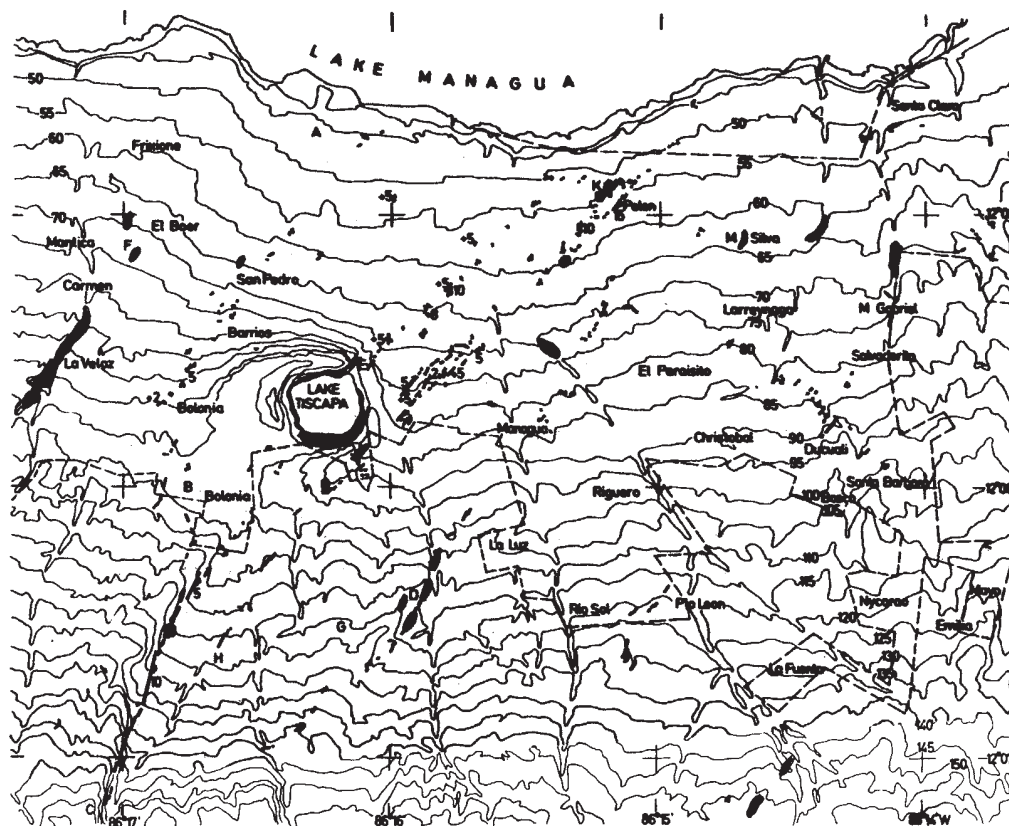


Fig. 1 Ground deformations associated with the earthquake of December 23, 1972, in Managua. Displacements in centimetres; contours in metres; shading indicates landslide and slumping areas. The city limits are shown by a thin dashed line and city districts are indicated. A, Cathedral; B, El Retiro General Hospital; C, Country Club; D, American College; E, US Embassy; F, Somoza Stadium; G, Universidad Centroamericana; H, Quinta Cecilia; K, Custom House; M, Cartographic Service.

techniques were adequate, construction was infested with bad detailing and materials. In most cases modern methods of design were not followed up by equally advanced construction methods.

The epicentral intensity assigned to the earthquake by various observers varies from VII to X (MM), and intensity maps, prepared by different field parties, seem to depict the distribution of the quality of building materials, methods of construction and age of buildings in the city rather than the distribution of earthquake intensity; no two samples of these maps look remotely alike.

The earthquake of 1972 was associated with minor but extensive ground deformations. These were found in surfaced roads, gardens and open spaces in the city as well as in the fields to the southeast of Managua. A cursory study of these features shows that most offsets on individual fractures were left-lateral by as much as 15 cm and that vertical offsets displayed very small throws, mainly to the southeast and not exceeding 5 cm. Individual fractures, arranged *en echelon*, did not extend uninterrupted for more than some tens of metres.

In Fig. 1 I show the ground fractures associated with the earthquake. These fractures define a rupture zone about 6 km long and 1.5 km wide which coincides with the Tiscapa fault. The cumulative lateral offset measured across this zone is at least 50 to 60 cm.

Long rigid elements, such as pavements with large concrete slabs, curbs and surfaced footpaths crossing the zone at an acute angle were in places found to be humped up and shortened or stretched out by 30 to 40 cm.

It is probable that this rupture zone extends northeast into Lake Managua. To the southwest, minor ground deformations and landslides found to the southeast of the 6th and 9th kilometre "carretera" of the Panamericana Motorway suggest that this zone, which trends N 38° E, extends for another 6 km in that direction.

This zone of fractures, about 12 km long and 1.5 to 2.0 km wide, seems to reflect the surface expression of a tectonic adjustment on the buried Tiscapa fault.

There seems to have been no change in the shoreline of Lake Managua, nor is there any indication of movement on the alleged rupture zone of 1931. Slumping of the ground in and around the Somoza Stadium was due to the collapse of underground galleries belonging to the old prison that occupied the site.

A detailed field study of the distribution of damage in the city, carried out by the writer on two separate occasions, shows that there was little, if any, relationship between damage due to vibrational effects and proximity to the surface ruptures.

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Search for Superheavy Elements in Natural and Proton-irradiated Materials

We have used an improved method of measuring low levels of spontaneous fission to establish limits for postulated superheavy elements in natural materials. The apparatus has also been used to give an approximate measure of the rate of cosmic ray-induced fission of lead. Our result for lead agrees with theoretical predictions and satisfactorily explains observations of fission events in Pb glass that had previously been attributed to superheavy element decay¹. Measurements on a lead target irradiated with 29 GeV protons were carried out as a comparison with the latest results of Marinov *et al.*², which would have predicted 15 counts in our experiment. Our sample yielded no counts.

The search for superheavy elements has been stimulated by theoretical work that indicates an island of nuclear stability in the region of the double shell closure at $Z=114$, $A=298$ (refs 3 to 5). Nilsson *et al.*⁶ originally predicted that the total half life for ²⁹⁴110 might exceed 10⁸ yr, with spontaneous fission as the decay mode of the superheavy or its daughter. Several experimental attempts have been made to detect the spontaneous fission of superheavy elements which might still be stored in natural materials⁷⁻¹⁰.

Our search used an ultra-low level counting scheme to detect the fission fragments from any superheavy nuclei present. The fission fragments were recorded as coincidence counts in two adjoining plastic detectors. The high sensitivity was achieved by the use of large areas and long exposure times; a typical sample (100 g) was counted for 3 months with a detection area of 1 m². The finely ground sample was spread on a perspex plate and placed next to a pair of 3.5 µm 'Kimfol' sheets (Peter S. Schweitzer Division, Kimberly-Clark Corporation, Lee, Massachusetts) mounted on separate plastic frames. The sample was separated from the two detector sheets by a 75 µm plastic sheet while counting was not in progress. The experiment was run in the Fairport Harbor Salt Mine, 585 m below the surface, to avoid induced fissions from cosmic rays. The protective sheets were withdrawn only while the sample-counter assemblies were in the mine.

The 3.5 µm sheets were later etched in NaOH to develop fission tracks, which were detected by a pulse spark method¹¹. The position of individual tracks was measured to ±1 mm. The background rate, which was due to flaws in the plastic, was 225 counts m⁻² for a single sheet and 4×10^{-3} counts m⁻² for two sheets in coincidence. The efficiency for detecting fission fragments from a thick depleted uranium source was determined to be $16.0 \pm 1.2\%$. Details of the sample preparation, etching, spark counting and calibration procedures will be published elsewhere.

We examined samples of the chemical homologues (Pb, Hg, Pt, . . .) of all possible superheavy elements from $Z=108$ to $Z=114$. Most were naturally occurring ores and minerals. Some were industrial concentrates. Finally a sample (area 1 inch square and length 2 inch) of galena irradiated by 9.4×10^{17} protons at 29 GeV/c, and one sample of purified thorium chloride (ThCl₄) were used.

Spontaneous fission fragments from uranium impurities constitute a background which contributes one fission event for a typical sample at uranium concentration of 8.8 p.p.m. The uranium concentration in each sample was measured by using neutron-induced fission recorded on mica detectors in conjunction with uranium glass standards¹². In about half the samples the uranium was highly non-uniformly distributed and, for these, a new method of average uranium determination based on track techniques was used (F. H. G., J. Shirck and R. M. W., to be published). The thorium sample was measured spectrophotometrically¹³ because fast neutron fission of thorium caused a large interference reaction. The thallium mine tailings and the thorium were unacceptably high in uranium. These samples were sent to Dr Johann Korkisch of the University of Vienna, who lowered the uranium concentration by an ion-exchange process.

Although there were minor variations among samples, most were activated for 3 months with an area of 1 m². Of the forty samples exposed deep underground thirty detected no fission events, eight detected one, and one each detected three and five. As a single track in any sample can be attributed to flaws in the plastic, and as the samples giving the high counts had an appropriate concentration of uranium, we consider that we have no evidence for the existence of a superheavy element.

Using an assumed half life of 10⁸ yr, we conclude that for a typical sample the concentration of superheavy elements is $<7.6 \times 10^{-18}$, $<4.5 \times 10^{-18}$ or $<2.9 \times 10^{-18}$ relative to the heavy homologue, assuming the range of the fission fragments is the same as for uranium¹⁴, or increased as predicted for symmetric fission or for asymmetric fission, respectively¹⁵.

The negative result of the superheavy search also implies that the half lives of Pb, Hg, and Th against spontaneous fission are $> 3.8 \times 10^{22}$ yr, $> 3.6 \times 10^{22}$ yr, and $> 2.4 \times 10^{20}$ yr respectively.

A galena (PbS) sample irradiated with 9.4×10^{17} protons at 29 GeV some 11 months before the start of counting recorded no spontaneous fission events. If the production rate of superheavy elements is the same for a PbS target as originally reported by Marinov *et al.*¹⁶ for a tungsten target, some fifty-one events would have been expected. Our negative result supports the later work of Marinov and others¹⁷⁻¹⁹. The null count for the proton irradiation also sets an upper limit of 3.6 pbarn for the production of ²⁸⁵Cf by the impact of 29 GeV/c protons on PbS.

The fission rate of galena was measured as 6.4 ± 5.3 and 9.6 ± 6.7 fissions $\text{g}^{-1} \text{yr}^{-1}$ at the surface in St Louis and at 200 g cm^{-2} below ground respectively. The expected induced fission rate computed using a calculated cosmic ray-induced atmospheric neutron spectrum given by Hess *et al.*²⁰, corrected for the latitude of St Louis²¹, and using energy-dependent fission cross sections given by Axmann *et al.*²² is 12 fissions $\text{g}^{-1} \text{yr}^{-1}$ for lead, in agreement with our experimental results.

Our value for the cosmic ray production rate is in agreement with that recently measured by G. N. Flerov, V. P. Perelygin and O. Otgonsuren (unpublished) and supports their contention that fossil fission tracks in ancient Pb glasses should be attributed to the effect of cosmic rays.

Our results imply either that the production rate of superheavy elements by normal r-process mechanisms is more difficult than calculated by Schramm and Fowler²³ or that the lifetimes are shorter than 10^8 yr. Although, of course, we may have looked in the wrong materials, it seems likely that a more, fruitful approach to the search for superheavy elements lies in studies of heavy ion reactions in targets with high atomic numbers.

We thank Claude Phillips, Bernice Franklin, and Andy Taube for their help with the low level counting scheme; Jim Boyle for discussions; Jerry Schlapper for the neutron irradiation of the samples; the Morton Salt Company for the use of the mine; and Matthey-Bishop, Inc., for the loan of a platinum sample. This work was supported by the National Science Foundation.

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Received May 21; revised July 9, 1973.

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Search for Superheavy Elements and Actinides Produced by Secondary Reactions in a Tungsten Target

We have presented evidence¹ for the possible existence of a superheavy element with atomic number 112. This evidence came principally from the observation of spontaneous fission activity in a mercury source separated from a tungsten target which had been irradiated by 24 GeV protons. It was subsequently shown² that at a later stage 70% of the fission activity was due to ²⁵²Cf. The origin of the remaining 30% of the fission activity was unexplained, however, although measurements of the activity made when the source was first produced indicated that it could not then have been due to ²⁵²Cf.

Our previous results were based on experiments performed with three different targets identified as W1, W2 and W3 respectively. Since then experiments have been carried out with two other targets, W4 and W5, and the results from these will be described. As our previous evidence was based on the possible production of eka-Hg, the chief aim of the present experiments has been to separate eka-Hg from the tungsten target. As well as looking for possible superheavy elements a search was also made for actinides, both as a group and individually, in order to test the possible reaction mechanism. A summary of the target irradiations is given in Table 1.

Table 1 Summary of Target Irradiations

	Weight of W rod (g)	Proton dose	No. of ¹⁴⁸ Gd atoms produced
W1	33	2×10^{18} *	
W2	33	7×10^{17} *	$6 \times 10^{15} \dagger$
W3	36§	1×10^{18} *	
W4	45	$9.1 \times 10^{17} \dagger$	$1.0 \times 10^{16} \dagger$
W5	45	$6.7 \times 10^{17} \dagger$	$7.6 \times 10^{15} \dagger$

* External beam monitor value, provided by CERN. Estimated error $\pm 20\%$.

† ²²Na used as an internal monitor, assuming a formation cross-section of 1.6 mbarn (refs 7 and 8).

‡ Calculation based on the measured formation cross-section of ¹⁴⁸Gd (29 ± 4 mbarn) (ref. 7).

§ The target was different from all others in that it was heavily oxidized to WO₃ (~28%).

The chemical separations carried out on the tungsten targets are described in detail elsewhere³. For the W4 target a Pt cathode and an excess of NaOH was used in the anodic dissolution vessel. A large fraction (20% to 95%) of the heavy metals (Os and Pb) was deposited as a thin layer on the cathode⁴. This was kept at liquid nitrogen temperature and counted for spontaneous fission events over a 9-d period using polycarbonate plastic detectors. No events were observed. Later experiments showed, however, that elementary Hg deposited on the cathode was lost at a rate of 50% a week even at liquid nitrogen temperature. Because eka-Hg is likely to be more volatile than Hg this experiment cannot be considered conclusive. For possible future experiments we should note that counting the cathode for spontaneous fission events could be a useful way of searching for other less volatile superheavy elements. This technique

involves a minimum of chemical separation and is therefore less likely to cause the loss of the wanted activity or to introduce contamination.

For the W5 target a Mo cathode was used with stoichiometric amounts of NaOH in the dissolution vessel². To prevent loss by evaporation, the activity on the cathode was removed immediately by reverse electrolysis³. Following chemical separation, the Hg source was made by exchange on a thin PbS film.

For both the W4 and W5 targets the separation of the actinide and lanthanide elements as a group was carried out in the conventional way using F⁻ and OH⁻ precipitations and anion exchange. The individual actinides from the W5 target were separated by the use of reverse phase partition chromatography using radioactive lanthanide isotopes to calibrate the column⁴. The sources were prepared electrolytically using stainless steel disks as cathodes. More than 95% of the transplutonium actinides were deposited in this way⁴.

Both the actinide sources and those of the elements Os to Pb from the W5 target were counted for spontaneous fission activity with polycarbonate plastic detectors but no such events were observed in 2 weeks; on the assumption that the superheavy elements decay by this mode with a half life of 1 yr the upper limit for their formation cross-section is 10⁻¹⁸ barn.

Alpha activity was measured using surface barrier detectors for varying periods from days to several weeks. No known transplutonium elements were observed in the actinide sources. Most of the alpha peaks found in the other sources were probably due to known isotopes such as ¹⁴⁴Gd, ²¹⁰Po and members of the ²³²Th decay chain.

Before comparing these results with those obtained from the earlier targets, the following changes in the chemical separation should be mentioned. In the present work NiS was used as a coprecipitant instead of MnO₂, because it was found to give higher and more reproducible yields of the elements Os to Pb. 'Dowex' 50 was used rather than 'Dowex' 1 to facilitate the separation of eka-Hg from thorium and lanthanide contamination. It is, of course, not certain that the eka element will follow its homologue in the initial or modified chemical procedures, and it is always possible that this could be the reason for the negative results of W5. This same argument also applies when comparing our work with that of other groups who have carried out similar experiments but using a different chemical approach (refs 5 and 6 and Yu. A. Batusov, Ts. Vylov, V. P. Trelov *et al.*).

For the actinides the comparison can be made more easily as the chemistry of this group of elements is well established. Our results are in agreement with those of Unik *et al.*⁵, Westgaard *et al.*, and Batusov *et al.* (unpublished). We now believe that the alpha activity previously observed in our actinide sources from W1 and W2 was largely due to contamination. These findings imply that the production of energetic fragments with $Z > 22$ to give secondary reactions in a tungsten target must be less than 3×10^{-18} barn. (Calculations were based on production of ²⁴⁴Cm.) In the case of uranium targets irradiated with 10 to 28 GeV protons, only reaction products with atomic numbers up to 6 greater than that of the target material were found⁶.

We are, however, still unable to explain the origin of the fission activity observed in the Hg source produced from the early W2 target. If the spontaneous fission activity had been due to ²⁵²Cf then at least 30 counts d⁻¹ would have been seen in the alpha particle spectra at an energy of 6.1 MeV. In fact, as can be seen from Fig. 1a and b of ref. 1, less than 12 counts were observed in this energy region in 27 d. Similarly, measurements of the alpha/fission ratio made using a single surface barrier detector gave an upper limit of 5:1. We have recently reanalysed this data and can find no explanation in terms of ²⁵²Cf or other known actinide isotopes. The origin of the fission events observed in the early stages of the W2 experiment remains unexplained.

In conclusion this experiment shows that we are unable to repeat the results obtained in the experiment of ref. 1. We do not observe any significant production of actinides, in agree-

ment with other work, indicating that the secondary reaction process is unlikely to be a useful way of producing superheavy elements. We believe that future experiments must rely on direct heavy ion reactions.

We thank A. Herz and Ch. Steinbach of CERN for providing irradiated tungsten targets, Mrs K. Glover from AERE for her assistance on many occasions, and the SRC for a research grant for technical assistance.

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Nature of "Anomalous Water"

MANY experiments have corroborated the phenomenon involving the formation of condensates with anomalous properties from the vapours of water and other liquids on silicate surfaces. But the nature of this phenomenon remained obscure for a long time and widely differing hypotheses were put forward to clarify it, one of them involving the formation of stable associates of water molecules (H₂O)_n. This hypothesis was first formulated by us¹⁻⁴ and was developed further by Lippincott *et al.*^{5,6}.

The principal argument in favour of this hypothesis was that the contact of liquid water with the same surface does not modify its properties appreciably. That is why it seemed impossible to explain that effect in terms of the simple dissolution of substrate or dissolution of the surface impurities. The "polywater" hypothesis seemed also to be corroborated by the results of primary analytical investigations that failed to detect impurity elements in it^{7,8}.

An alternative hypothesis attributed the anomalous properties of condensates to the effect of soluble impurity compounds detected experimentally during subsequent investigations. Yet there was no consistent opinion, either with regard to the mechanism of their getting into the condensate or with regard to their respective compositions. This resulted both from differing conditions in various laboratories producing "anomalous water" and from the technical difficulties involved in the analysis of extremely small amounts of a substance⁹⁻¹³.

We have succeeded only quite recently in overcoming these difficulties and achieving fairly convincing results. First, in collaboration with V. I. Spitsin and his co-workers, we used a neutron activation analysis method¹⁴ to detect an increased concentration of sodium and silicon atoms in the anomalous water samples. Then we used an electron probe method¹⁵ to carry out investigations that were accompanied by an estimation of the layer thickness of a substance being analysed¹⁶. This was needed to exclude errors due to the incomplete absorption of electrons by a sample under examination. Samples of "anomalous condensates" as produced by many different methods were examined.

We have established that there are no condensates both free of impurity atoms and simultaneously exhibiting anomalous properties. Consequently, these properties should be attributed to impurities rather than to the existence of polymeric water molecules. In all probability, the anomalous component is in general a mixture of silicic acid colloidal particles and molecular-dissolved compounds of Na, Si, O, C, K, Cl, and S. The particles of a sol converting itself on evaporation in a gel impart increased viscosity, density, and refractive index to anomalous condensates.

The molecular-dissolved compounds reduce both the vapour pressure of a solvent and its melting point. The chief part of the anomalous condensate is amorphous, sometimes containing small amounts of KCl and NaCl in crystalline form¹⁷. When produced in quartz capillaries under the cleanest conditions attainable, the samples chiefly contain O, Si, and Na. This may be explained by the enrichment of quartz surface layers with sodium impurities when drawing out the capillaries. An increase in the sodium concentration in the condensate may help to enhance the solubility of quartz. Yet, when introducing liquid water into similar capillaries, the amount of silicon and sodium atoms dissolved was always found to be much smaller. This indicates that the dissolving power of freshly condensed water is higher.

Attempts to produce typical anomalous condensates on a thoroughly washed, polished surface of quartz (not enriched with sodium) have failed. Experiments were carried out *in vacuo*, and a vapour stream in the form of a molecular beam scanned an area of 25 mm², creating an abrupt change in the vapour pressure at each point. The matter washed off from the working area contained only silicon and oxygen in the same relative proportions as found in the standard silicic acid gel. An aqueous solution of that substance exhibited only some of the characteristic properties of anomalous water (high density, refractive index and viscosity, for example). Hence, a pulsing vapour pressure and periodic condensation serve to increase the colloidal dissolution of quartz.

In the case of samples not obtained under strictly "sterile" conditions, the presence of atoms of K, C, Cl and S may rather be attributed to the surface diffusion of impurities from an ambient medium. Rousseau¹⁸ proved that this mechanism can increase the content of impurities in condensates.

Consequently, the anomalous properties of condensates may be explained, not by the formation of a new modification of water, as was previously supposed, but by the peculiar features of a reaction taking place between the vapour and solid surfaces in the process of condensation. Many aspects of the mechanism of formation of anomalous condensates have not yet been fully clarified. This especially applies to the formation of anomalous condensate on MgO surfaces^{19,20}. Only the general features of the phenomenon are clear as yet; thorough investigation by those studying processes involving the interaction of vapours and solid surfaces is clearly required.

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Second Law of Thermodynamics

WILKIE¹ has recently proposed a statement of the Second Law of Thermodynamics which he implies is both new and simple:

"The only processes that can happen spontaneously are those that can in principle be made to yield work, for example, to lift up a weight."

In fact this is not original; nor is it a statement of the Second Law. A similar but more extensive statement, made more meaningful by an additional sentence, was put forward in a book some years ago by J. A. V. Butler²:

"Spontaneous processes (that is, the processes which may occur of their own accord) are those which when carried out under proper conditions, can be made to do work." Butler then adds, "If carried out reversibly they will yield a maximum amount of work; in the natural irreversible way the maximum work is never obtained."

Butler uses the first sentence merely to define "spontaneous processes" and the second to place a limitation on the work. Thus Wilkie's statement is original only in that it lacks the additional sentence. All it says is that something that is happening can be made to do useful work.

If a statement of the Second Law of Thermodynamics is to be both a proper and useful alternative to the conventionally accepted statements it must satisfy two requirements. First, it must possess the universality of the conventional statements, otherwise it is not a statement of the law and second it must lead to the usual criteria of equilibrium and all of the thermodynamic relationships which follow from the conventional statements.

Wilkie's statement is lacking in both of these requirements. Clearly, no attempt has been made to show how the criteria of equilibrium, and all the thermodynamic relationships, follow from it; indeed it is difficult to see how this can be done without at least an additional sentence similar to that of Butler.

The most serious criticism of Wilkie's restatement of the Second Law is, however, in respect of the first condition

mentioned above, that is, the question of its universality. One may quote Planck³ as an authority here. After defining entropy and indicating how to measure entropy changes (parts of the statement of the Second Law), Planck states:

"Every physical or chemical process in nature takes place in such a way as to increase the sum of the entropies of all the bodies taking part in the process. In the limit, that is, for reversible processes, the sum of the entropies remains unchanged. This is the most general statement of the Second Law of Thermodynamics. . . . It should be emphasized, however, that the form here given is the only one of unrestricted applicability to any finite process, and that no other universal measure of the irreversibility of the processes exists than the amount of the increase of the entropy to which they lead. All other forms of the Second Law are either applicable to infinitesimal changes only, or presuppose, when extended to finite changes, the existence of some special conditions imposed on the process."

Now Wilkie proposes a "general proof" that the accepted criterion of spontaneity, $\Delta S_{cr} > 0$, is the same as his criterion that the system be capable of performing work. In support of his claim he uses the equation

$$\Delta S_{cr} = (W_{max} - W)/T_c$$

which purports to relate generally the entropy created as a result of a change in a non-isolated system contained in an environment at temperature T_c to the work actually obtained, W , and the maximum work possible, W_{max} . Such an equation is not general and at best applies only if the temperature of the environment is constant (if indeed it is ever true for other than the trivial case for which the temperature T_c of the environment is equal to the temperature T of the system throughout the process). Moreover, the derivation of the equation, whatever the conditions for which it is valid, must begin from a conventional statement of the Second Law.

Thus all that Wilkie has shown is that, in the special case of a process in a large constant temperature environment, his statement is contained within the conventional statement of the Second Law (which we note says more than just that $\Delta S_{cr} > 0$ for a spontaneous process). Wilkie has not shown that the conventional statement is contained within his and hence it does not follow that the two statements are equivalent. Indeed, Planck (ref. 3, page 104) comments:

"The amount of 'lost work' yields a no more definite general measure of irreversibility than does that of 'dissipated energy'. This is possible only in the case of isothermal processes."

Wilkie's statement further lacks universality in that it cannot deal with processes in isolated systems. Clearly, an irreversible process in such a system can, by definition, do no work. In an attempt to circumvent this difficulty Wilkie states "this argument can be extended to an isolated system: in that case the work resulting from the spontaneous change must be stored as such within the system". Now work is a mode of transfer of energy⁴ and it is meaningless to claim that a mode of transfer of energy can be stored as such within the system. It could possibly be argued that a process occurring in an isolated system could cause the temperature inside the isolated enclosure to increase and that "work" is thereby stored until the system is broken into and the temperature difference between the inside and outside of the enclosure used to do work. Even this loose usage does not, however, lead to generality. For example, consider two different perfect gases, capable of forming a perfect gas mixture, both at the same temperature and pressure but separated by a partition in a thermally and mechanically isolated enclosure. When the partition is removed the gases spontaneously mix to give a final state in which the total pressure, volume, temperature and internal energy are unchanged. It would be interesting to know how the work is stored in the final state of this isolated system.

In conclusion, Wilkie's statement of the Second Law of

Thermodynamics is not original, lacks generality, is inapplicable to isolated systems and has not been shown to lead to the usual thermodynamic relationships. It is clearly unacceptable as an alternative to the conventional statements.

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BIOLOGICAL SCIENCES

Inhibition of Rat Liver RNA Synthesis by Aflatoxin B₁

THE administration to rats of aflatoxin B₁, a potent hepatotoxin and carcinogen, rapidly results in an inhibition of both hepatic RNA synthesis *in vivo*¹ and Mg^{2+} and Mn^{2+} — $(NH_4)_2SO_4$ stimulated RNA polymerase activities, assayed *in vitro*^{2,3}. Previous work has demonstrated that the RNA polymerase enzyme, isolated from hepatic nuclei whose *in situ* polymerase activity was inhibited by previous administration of aflatoxin B to the animals, has an activity equal to that of enzyme isolated from control tissues^{3,4}. The enzyme was solubilized by incubating the nuclei in a low-salt medium, which predominantly extracts the nucleolar polymerase enzyme. We have now carried out further experiments using the more vigorous technique of ultrasonication in a high-salt medium⁵ which solubilizes both the nucleolar and nucleoplasmic enzymes, and have separated the enzymes by gel filtration⁶.

Table 1 shows that, whereas both the Mg^{2+} and Mn^{2+} — $(NH_4)_2SO_4$ stimulated polymerase activities present in intact nuclei are strongly inhibited by the *in vivo* administration of aflatoxin B₁, the polymerase enzymes extracted by the high-salt technique retain the full activity of the nucleolar enzyme. Like Saunders *et al.*⁷, we observed a decrease in the activity of the unfractionated enzyme extract assayed in the presence of Mn^{2+} — $(NH_4)_2SO_4$, which was reflected in a decrease in activity of the nucleoplasmic enzyme peak eluted from the DEAE-'Sephadex' column (Fig. 1). In contrast to the results of Saunders *et al.*⁷ the percentage inhibition of the isolated nucleoplasmic enzyme was consistently less than that of the Mn^{2+} — $(NH_4)_2SO_4$ stimulated *in situ* polymerase activity (Table 1). The decreased activity of the nucleoplasmic enzyme was not accompanied by any quantitative changes in the relative activities of the enzyme when Mn^{2+} or Mg^{2+} were present as cofactors nor in the stimulation of activity which resulted from the addition of ammonium sulphate (Table 2). The dependence of the activity of the enzymes on the concentration of divalent cations in the assay medium was also unchanged (Fig. 2). This situation resembles the action of α -amanitin on RNA synthesis.

To explain the action of α -amanitin it has been suggested that a product of the nucleoplasmic polymerase activity regulates the synthesis of another RNA species, either directly through the RNA it produces or indirectly through a protein formed by the translation of a fraction of the RNA produced⁸. Certain differences exist, however, between the

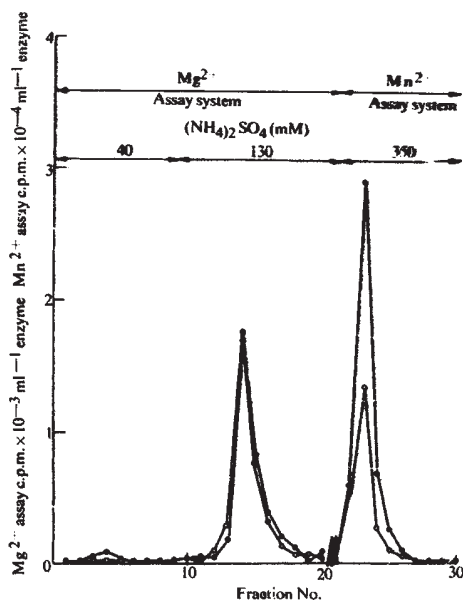


Fig. 1 Fractionation of nucleolar and nucleoplasmic polymerase enzymes on DEAE-Sephadex. 6.68 mg protein placed on a DEAE-Sephadex column (2×3.25 cm) eluted with TGMED solution, first 27 ml containing 40 mM (NH₄)₂SO₄, followed by 27 ml containing 135 mM (NH₄)₂SO₄ then 36 ml containing 350 mM (NH₄)₂SO₄. 3 ml fractions were collected. Assays were carried out as in Table 1. Enzymes prepared from 16 g pooled liver from groups of 5 male Fischer rats, average wt 100 g. One group was injected with aflatoxin B₁ (10 mg kg⁻¹) in dimethylsulphoxide. The other groups received the vehicle alone. Rats were killed 2 h after injection. ●, Control; ○, aflatoxin treated.

actions of α -amanitin and aflatoxin B₁ which render such an explanation unlikely in the case of the latter. α -Amanitin has a marked differential inhibitory action on the activities of the Mg²⁺ and Mn²⁺ ammonium sulphate polymerases in intact nuclei (20% maximum inhibition compared with 70%)⁸ whereas aflatoxin B₁ has an equal effect on the two activities. The rapid (<5 min) effect of aflatoxin B₁ on nuclear polymerase activity, affecting both polymerase activities equally³, does not suggest that inhibition of the Mg²⁺ stimulated activity depends on a previous inhibition of the Mn²⁺—(NH₄)₂SO₄ stimulated activity. It seems, therefore, that inhibition of RNA synthesis by aflatoxin B₁ and

α -amanitin, although superficially similar, depends on different underlying mechanisms, and that a better comparison for the action of aflatoxin B₁ is actinomycin, which inhibits both Mg²⁺ and Mn²⁺—(NH₄)₂SO₄ activated polymerase through an action on the template mechanism. Supporting this explanation is the rapid effect of both aflatoxin B₁ and actinomycin on nucleolar morphology which has been found to be temporally associated with the inhibition of rRNA synthesis *in vivo*². We have examined the effect of *in vivo* treatment with aflatoxin B₁ on the template capacity of rat liver chromatin and DNA, and the inhibition of the template capacities of chromatin and native DNA can be seen from the results given in Table 3. The reduction in inhibition upon heat denaturing the DNA (Table 3) could presumably be due either to the removal of the inhibitory substance by heating or to the increased availability of initiation points due to the denaturing process.

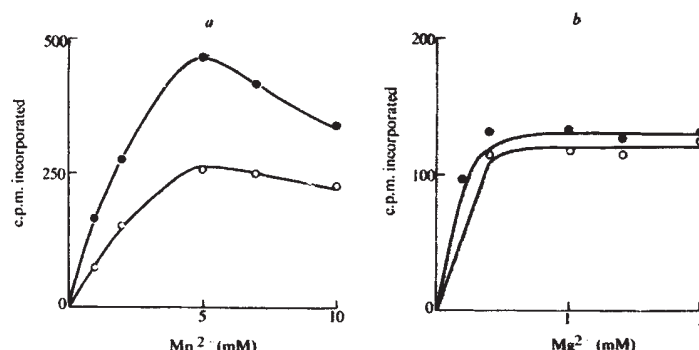


Fig. 2 *a*, Dependence of nucleolar polymerase enzyme, isolated from control and aflatoxin B₁ treated rats, on Mg²⁺ concentrations. Assay conditions as in Table 1. ●, Control enzyme; ○, enzyme from aflatoxin B₁ treated animals 2 h before killing. Enzyme fractions 11 to 14 (Fig. 1) combined. *b*, Dependence of nucleoplasmic enzyme, isolated from control and aflatoxin B₁ treated rats, on Mn²⁺ concentration. Assay conditions as in Table 1. ●, Control enzyme; ○, enzyme from aflatoxin B₁ treated animals 2 h before killing. Enzyme fractions 21 to 24 (Fig. 1) combined.

The failure of aflatoxin B₁ to inhibit the template capacity of DNA (or the activity of the polymerase enzymes) when added to *in vitro* systems may be due to a requirement for metabolic conversion of the aflatoxin B₁ to a reactive product. The microsomal fraction of rat liver homogenates has

Table 1 The Effect of Aflatoxin B₁ on RNA Polymerase Activity

	Assay medium	Treatment	Polymerase activity C.p.m. incorporated per mg DNA $\pm$ s.e.	B ₁ treated as % of dimethylsulphoxide controls
Intact nuclei	Mg ²⁺	Dimethylsulphoxide	5,657 $\pm$ 90	
		B ₁	1,219 $\pm$ 118	22
	Mn ²⁺ + (NH ₄) ₂ SO ₄	Dimethylsulphoxide	8,074 $\pm$ 333	
		B ₁	1,817 $\pm$ 72	23
			C.p.m. incorporated per 50 μ l enzyme (134 μ g protein)	
Unfractionated solubilized enzyme	Mg ²⁺	Dimethylsulphoxide	549 $\pm$ 5	
		B ₁	594 $\pm$ 12	108
	Mn ²⁺ + (NH ₄) ₂ SO ₄	Dimethylsulphoxide	913 $\pm$ 64	
		B ₁	397 $\pm$ 10	43

Assay conditions for: intact nuclei, Mg²⁺: 50 μ mol Tris-HCl, pH 8.5, 2.5 μ mol MgCl₂, 10 μ mol cysteine, 3 μ mol NaF, 30 μ mol ATP, CTP, GTP, 20 nmol ³H-UTP (0.5 μ Ci), 15 min incubation at 37° C; for Mn²⁺—(NH₄)₂SO₄: 50 μ mol Tris-HCl, pH 7.5, 2 μ mol MnCl₂, 0.5 M (NH₄)₂SO₄. Nucleotides as in Mg²⁺, 10 min incubation in the absence of nucleotides, followed by 30 min incubation in the presence of nucleotides. Solubilized enzyme, Mg²⁺: 50 μ l enzyme in TGMED (50 mM Tris-HCl, pH 8, 25% v/v glycerol, 5 mM MgCl₂, 0.1 mM EDTA, 0.5 mM dithiothreitol), 50 μ g highly polymerized calf thymus DNA, 40 μ mol Tris-HCl, pH 8.0, 2 μ mol NaF, 2.5 μ mol dithiothreitol, 5 mM MgCl₂. Nucleotides as above, 30 min incubation. Mn²⁺—(NH₄)₂SO₄: 50 μ l enzyme in TGMED, 50 μ g heat-denatured calf thymus DNA, 40 μ mol Tris-HCl, pH 8.0, 2 μ mol NaF, 2.5 μ mol dithiothreitol, 1.0 mM MnCl₂, 88 mM (NH₄)₂SO₄. Nucleotides as above, 30 min incubation. Animals and injections as in Table 3.

Table 2 Comparison of Cofactor Requirements of Nucleoplasmic Enzymes Isolated from Livers of Control and Aflatoxin B₁ Treated Rats

	RNA polymerase activity c.p.m. per 200 µl enzyme		$Mg^{2+}/Mn^{2+} \times 100$ $+(NH_4)_2SO_4 \times 100$ $-(NH_4)_2SO_4 \times 100$
	Mg^{2+}	Mn^{2+}	
Undialysed			
Control	165 ± 8	1,188 ± 49	14%
Aflatoxin	115 ± 4	569 ± 20	20%
Dialysed	$-(NH_4)_2SO_4$	$+(NH_4)_2SO_4$	
Control	406 ± 23	635 ± 22	156%
Aflatoxin	168 ± 6	271 ± 5	161%

Enzymes prepared as in Fig. 1. Assay conditions for undialysed enzyme assays, Mg^{2+} and Mn^{2+} cofactors as in isolated enzyme assays in Table 1 except $(NH_4)_2SO_4$ concentration 88 mM in both Mg^{2+} and Mn^{2+} assays. Dialysed enzyme assays, nucleoplasmic enzyme after elution from column with 350 mM ammonium sulphate (Fig. 1) dialysed overnight against 11 TGMED to remove ammonium sulphate. Assays carried out with Mn^{2+} as in isolated enzyme assays in Table 1. Assays with $(NH_4)_2SO_4$ contained in addition 88 mM $(NH_4)_2SO_4$.

been shown to possess aflatoxin B₁-metabolizing activity⁹. We have examined the metabolism of aflatoxin B₁ by rat microsomal systems and in common with other workers^{9,10} have found that hydroxylation to aflatoxin M₁ represents a major route. From previous studies, however, it would seem that hydroxylation is not the significant pathway in terms of the inhibition of RNA synthesis. Activation of the liver drug metabolizing systems by pre-treating the animals with phenobarbitone, in spite of increasing the overall metabolism of aflatoxin B₁ and the formation of aflatoxin M₁⁹, results in a decreased inhibitory effect of the toxin

Table 3 Effect of Aflatoxin B₁ Administered *in vivo* on RNA Polymerase Activity of Intact Hepatic Nuclei and Template Activity of Chromatin and DNA

		C.p.m. incorporated per mg DNA	B ₁ treated as a % of dimethylsulphoxide controls
Intact nuclei	Mg^{2+} Control	3,170 ± 287	
	B ₁	1,035 ± 94	33%
	Mn^{2+} Control	6,073 ± 423	
	B ₁	1,929 ± 190	32%
		C.p.m. Incorporated per ml nucleoplasmic enzyme	
Chromatin	Control	940 ± 40	
	B ₁	380 ± 30	40%
Double stranded DNA	Control	5,110 ± 150	
	B ₁	2,950 ± 90	58%
	Calf thymus	4,400 ± 40	
Heat denatured DNA	Control	10,750 ± 240	
	B ₁	9,740 ± 210	91%
	Calf thymus	9,600 ± 200	

Two groups of male Fischer rats, 50 to 75 g, were used. One group was given aflatoxin B₁ 10 mg kg⁻¹ in dimethylsulphoxide intraperitoneally and the other group received vehicle alone. They were killed 2 h later, the nuclei isolated³ and chromatin¹⁴ and DNA¹⁵ prepared. Nucleoplasmic enzyme was prepared from control animals as in Fig. 1. Assay conditions of intact nuclei are Mg^{2+} assay as in Table 1. Chromatin primed assays contained 35 µg DNA. DNA primed assays contained 20 µg DNA. Incorporation in absence of added primer (<50 c.p.m.) subtracted from results. Results are means of at least triplicate assays of duplicate samples ± s.e. Assays were primed with 20 µg calf thymus DNA included for comparison.

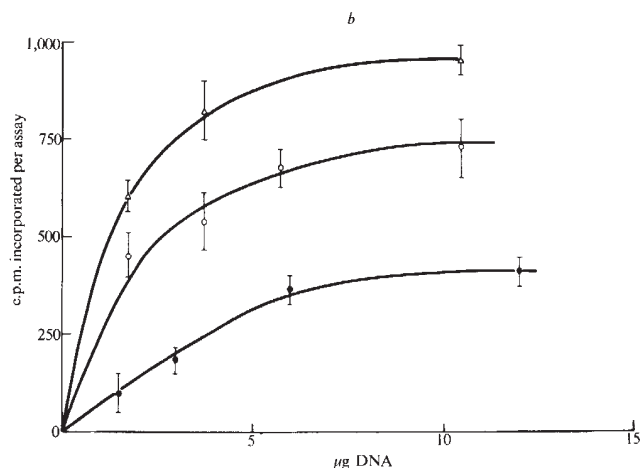
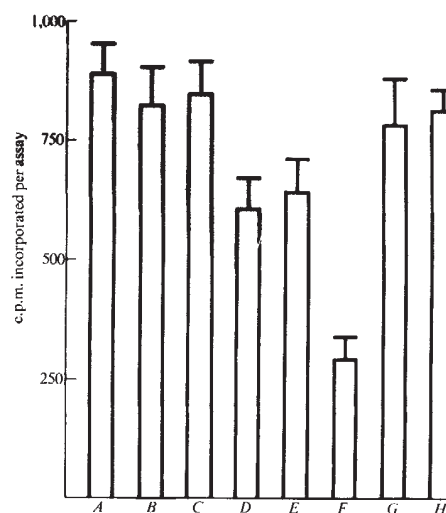


Fig. 3 *a*, Effect of microsome metabolism of aflatoxin B₁ on the template capacity of DNA. Washed microsomes were prepared by the method of Omura and Santo¹⁶. Complete microsome incubations (4 ml) contained 6 mg microsomal protein, 0.25 mM NAD, 0.22 mM NADP, 4 mM glucose-6-phosphate, 5 µg glucose-6-phosphate dehydrogenase, 1 mg highly polymerized calf thymus DNA, 50 µg aflatoxin B₁, 50 µl dimethylsulphoxide, 10 mM Tris-HCl, pH 8.0. Incubation was in the dark for 15 min at 37° C in a shaking water bath. After incubation they were centrifuged at 100,000g for 1 h. Supernatant was removed and DNA precipitated by adding the supernatant to 1 ml ice-cold spermine solution (15 mg ml⁻¹) DNA spooled onto fine glass rods, blotted, and redissolved in 3 ml 10 mM Tris-HCl, pH 8.0, by the addition of 0.3 ml saturated ammonium sulphate solution. The solution was dialysed overnight against 1 : 10 mM Tris-HCl, pH 8.0. Polymerase enzyme incubations carried out with microsomes and cofactors as above. Incubated enzyme used directly after high speed centrifugation to remove microsomes. Polymerase assays carried out using nucleoplasmic enzyme. Assay medium as for Mn^{2+} assay in Table 1, 15 µg DNA per assay. *b*, Effect of microsome metabolism of aflatoxin on the template capacity of a range of concentrations of DNA. Assay conditions as in Fig. 2*a*. Δ , DNA preincubated + aflatoxin B₁ as in C in *a*. $\circ$, DNA preincubated with dimethylsulphoxide + microsomes + cofactors as in E in *a*. $\bullet$, DNA preincubated + aflatoxin B₁ + microsomes + cofactors in F in *a*. Results are means of triplicate assays ± s.e.

administered *in vivo* on both the *in vivo* synthesis of RNA¹¹ and the activity of the RNA polymerases assayed in isolated nuclei *in vitro*¹².

We have examined the possibility that another metabolite of aflatoxin B₁ produced by microsomal systems might be involved in the inhibition of rat liver RNA synthesis by allowing the metabolism to proceed in the presence of DNA. Figure 3a shows that a decreased template capacity resulted from incubating the DNA with microsomes+NADPH generating system alone. The reason for this is not known, but it seemed to be dependent on microsomal metabolism as it was virtually eliminated if the cofactors were omitted from the incubations. In spite of the decreased template capacity due to microsomes+cofactors alone, a further inhibition of the template capacity was apparent when microsomal metabolism of aflatoxin B₁ had proceeded in the presence of the DNA. No comparable effect was observed when the aflatoxin B₁ was replaced by the dimethylsulphoxide vehicle alone. To observe the effect, it was found necessary to have the DNA present during metabolism. If aflatoxin B₁ was metabolized in the absence of DNA, and the DNA then added to the supernatant after spinning down the microsomes, no effect on the template capacity was observed. Experiments in which the nucleoplasmic polymerase enzyme was incubated with aflatoxin B₁ and microsomes did not indicate that a reduced enzyme activity resulted. This is in contrast to the recent finding of Moulé and Frayssinet¹³, who reported that an unidentified microsomal metabolite of aflatoxin B₁ can inhibit the activity of bacterial RNA polymerase *in vitro*. The reason for this difference is at present unknown and is being investigated.

The inhibitory action of aflatoxin B₁ *in vivo* on hepatic RNA synthesis is accompanied by a reduced template capacity of the chromatin. Such a mechanism could provide a basis for the equal inhibition by aflatoxin B₁ administered *in vivo* for the Mg²⁺ and Mn²⁺—(NH₄)₂SO₄ stimulated polymerase activities observed in intact isolated nuclei. The inhibition seems to require the metabolism of aflatoxin B₁ to an unstable metabolite by microsomal drug metabolizing systems. One explanation of the apparent inhibition of the nucleoplasmic enzyme is that the effect of aflatoxin on the template might result in a greater difficulty in releasing the nucleoplasmic enzyme from the chromatin.

I thank David Judah for skilled technical assistance.

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Received February 26; revised June 22, 1973.

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Peptide Formation mediated by Cyanate

CHEMICAL reactions of the sort that might have produced peptides from amino acids on the primitive Earth can be catalysed by kaolinite¹, montmorillonite², hydroxyapatite³⁻⁵ and condensed phosphates⁶⁻⁸. Hydroxyapatite has been suggested as a source of condensed phosphates because it yields pyrophosphate when reacted with cyanate⁹. For this reason, and because phosphates polymerize amino acids, we have investigated whether peptide formation can be enhanced by cyanate added to hydroxyapatite or orthophosphates. After we reported our preliminary results (at the national meeting of the American Chemical Society in Washington DC in September 1971), Choughuley *et al.*¹⁰ confirmed that orthophosphates catalysed the condensation of amino acids to peptides.

Hydroxyapatite was prepared and aged in solution as outlined by Perloff¹¹ and the crystal phase was identified by X-ray diffraction. The surface area was 50 m² g⁻¹ as measured by nitrogen adsorption using the Brunauer, Emmett and Teller technique (BET). Reagent grade chemicals were used for the other phosphate phases (Table 1).

Mixtures of hydroxyapatite and glycine, with and without cyanate, were prepared by combining 1 M solutions of the corresponding reagents. The mixtures were shell frozen and lyophilized. Equal aliquots were weighed, sealed in 'Pyrex' test tubes, and heated at 95° C for varying times. Attempts to condense glycine using hydroxyapatite or orthophosphates in aqueous solutions at 95° C for 10 days with and without cyanate failed. The aqueous reactions were prepared by combining 10 ml each of 1 M solutions of glycine, pH 6.1; KCNO, pH 9.2; hydroxyapatite, pH 5.3; and orthophosphate (pH ranged between 2.8 and 12.5, depending on the orthophosphate used). These solutions were used for the solid state reactions. The same procedure was followed with mixtures of various orthophosphates and glycine, with and without cyanate. The reaction products were dissolved in water and aliquots analysed for linear glycine peptides on an automatic amino acid analyser, using the methodology for neutral and acidic amino acids from protein hydrolysates¹².

The synthesis of diglycine was verified by isolating the material eluted at 140 min, desalting it on 'Dowex'-50 (H⁺ form) and comparing the isolated sample with the acid (6 N HCl, 110° C, 22 h) and enzymatically (leucine-amino-peptidase¹³) hydrolysed aliquots. The unhydrolysed sample gave a chromatographic peak that coincided with that of authentic diglycine and an equal aliquot of the acid-hydrolysed sample gave the theoretical yield of glycine to within 5%. Enzyme hydrolysis of a third aliquot reduced the amount of diglycine with a concomitant release of glycine. Analysis by gas chromatography¹⁴ of the N-trifluoroacetyl methyl ester derivative of the diglycine in the unhydrolysed

Table 1 Condensation of Glycine to Diglycine and Triglycine with Orthophosphates

Orthophosphate	Diglycine yield (%)	
	A	B
Na ₂ HPO ₄	0	1.85
Na ₂ PO ₄ ·12H ₂ O	5 (1.4)	0
NaH ₂ PO ₄ ·H ₂ O	0.5	7.35 (2.85)
NH ₄ H ₂ PO ₄	0	1.6 (1.0)
(NH ₄) ₂ HPO ₄	0	0
K ₂ HPO ₄	10.5 (3.9)	0.2
CaHPO ₄	0	0.14
Ca(H ₂ PO ₄) ₂ ·H ₂ O	7.0	1.9

Composition: A, orthophosphate (10⁻³ M), glycine (10⁻³ M), B, orthophosphate (10⁻³ M), glycine (10⁻³ M), KCNO (10⁻³ M). The reactions were carried out in hypohydrous conditions for 20 d at 95° C. The percentage of triglycine formed is shown in parentheses.

fraction further confirmed that diglycine had been synthesized in the reactions studied.

Diglycine yields obtained with hydroxyapatite and sodium dihydrogen orthophosphate as a function of time are shown in Figs 1 and 2. The results show that glycine can be condensed to diglycine with hydroxyapatite or sodium dihydrogen orthophosphate in the absence of cyanate. However, dipeptide yields were enhanced two to three-fold in the presence of cyanate.

Because cyanate enhanced diglycine formation, we examined its action in combination with the orthophosphates shown in Table 1. The results show that, with the exception of NaH_2PO_4 , the condensing action of cyanate with Na_2HPO_4 , K_2HPO_4 , and CaHPO_4 was, at best, marginal and actually suppressed the yields of diglycine with Na_3PO_4 , K_2HPO_4 and $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$.

The partial transformation of orthophosphate to pyrophosphate at 126°C under anhydrous conditions¹⁵ suggested that the polymerization of glycine at 95°C was related to polyphosphate formation. To test this hypothesis, ^{32}P -labelled $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ was added to $200\ \mu\text{l}$ of $0.1\ \text{M}$ NaH_2PO_4 and $100\ \mu\text{l}$ of $0.1\ \text{M}$ KCNO was added to half of this solution. Both solutions were lyophilized, sealed and heated for 6 d at 95°C . Each sample was dissolved in water and aliquots chromatographed on cellulose sheets using the solvent of Fuchs and Czeck¹⁶. Spots located by autoradiography were excised and radioactivity was measured in a liquid scintillation counter.

NaH_2PO_4 gave 2% pyrophosphate and 24% when cyanate was added. This yield may include carbamyl phosphate, resulting from the reaction of orthophosphate with cyanate¹⁷, and not resolved from pyrophosphate with the solvents used. These results suggested that the production of higher yields of diglycine with $\text{NaH}_2\text{PO}_4 + \text{KCNO}$ was catalysed by pyrophosphate or carbamyl phosphate. This possibility was tested by reacting equimolar dry mixtures of glycine with pyrophosphate and carbamyl phosphate for 6 and 10 d respectively at 95°C . No diglycine (or larger peptides) was formed by pyrophosphate or carbamyl phosphate.

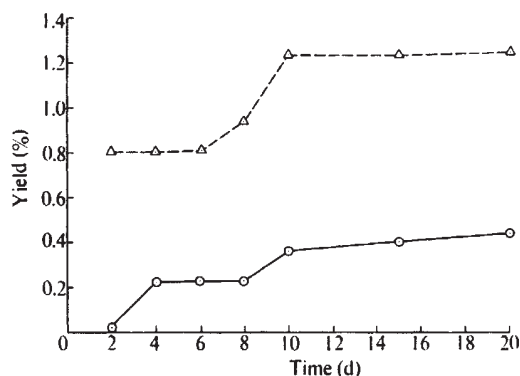


Fig. 1 Condensation of glycine to diglycine with apatite at 95°C . Δ , Equimolar mixture (0.25 mmol each) of glycine, apatite and potassium cyanate. $\circ$, Equimolar mixture (0.25 mmol each) of glycine and apatite.

The possibility that tripolyphosphate participated in the dimerization of glycine was explored by heating the anhydrous orthophosphates alone at 95°C for 20 d and assaying the thin-layer chromatography. None of the orthophosphates that effectively dimerized glycine condensed to tripolyphosphate or higher linear phosphates.

We believe that the orthophosphates in the absence of cyanate directly phosphorylated the carboxyl group of glycine and the glycine phosphate intermediate readily condensed with glycine. Analogous reactions involving acylphosphate intermediates have been reported¹⁸⁻²¹. Open or cyclic

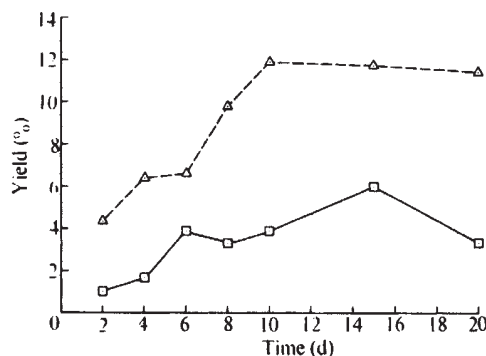


Fig. 2 Condensation of glycine to diglycine and triglycine with orthophosphate at 95°C . Percentage yield includes triglycine. Δ , NaH_2PO_4 ($3.5 \times 10^{-3}\ \text{M}$), glycine ($10^{-3}\ \text{M}$) and KCNO ($2 \times 10^{-3}\ \text{M}$). $\square$, NaH_2PO_4 ($3.5 \times 10^{-3}\ \text{M}$), glycine ($10^{-3}\ \text{M}$).

N-phosphoglycine cannot be excluded as an intermediate. Related reactions have been reported²².

Our results show that when glycine is heated in the presence of apatite or orthophosphates, diglycine is formed. The addition of inorganic cyanate increases the yields of diglycine but its action as a condensing agent extends only to some of the orthophosphates studied. Even though glycine is effectively dimerized with $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$, $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, K_2HPO_4 , and $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ there is reason to doubt the prebiotic significance of these reactions as these orthophosphates do not occur extensively in nature²³. We believe, however, that the reactions described here with hydroxyapatite and cyanate constitute a plausible model for condensation reactions on the primitive Earth, as apatite is the most thermodynamically stable solid calcium phosphate phase and is the form of phosphate most often found in nature. Furthermore, cyanate could be reasonably considered a prebiotic compound as it is a derivative of urea which in turn is synthesized from methane, ammonia and water by electric discharges²⁴ or high energy electrons²⁵.

We thank Sister Mary Kelly for technical assistance.

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Received February 9; revised May 10, 1973.

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Mechanical Instability of the Oxy-form of Sickle Haemoglobin

MUCH attention has been given to the molecular aspects of the deoxy form of sickle haemoglobin (Hb S) because sickling occurs with Hb S in the deoxygenated state. The oxy form of Hb S, on the other hand, is believed to be similar to normal adult haemoglobin (Hb A) with respect to solubility^{1,2}, X-ray diffraction pattern³, oxygen binding⁴ and other properties. During determination of oxygen equilibrium curves of Hb S, we noticed that a solution of oxyhaemoglobin S became turbid very easily when it was stirred or mixed. The turbidity was found to be due to protein which was denatured by the mechanical force involved and/or by foaming. Oxyhaemoglobin S denatured more quickly than oxyhaemoglobin A by mixing, stirring or sonication. The rate of denaturation was dependent on the oxygen concentration in the medium, suggesting that an oxidative process was involved. As demonstration of denaturation requires only a few drops of blood, water and shaking, this property can be used as a simple clinical test for the presence of Hb S.

Haemoglobin solutions were prepared from heparinized blood⁵ and used without further purification. Haemoglobin solutions from normal (A), sickle cell disease (S) and sickle cell trait (AS) individuals were diluted into 2.5 ml of buffer to make a final haem concentration of about 60 μ M. The solution in a test tube (150 $\times$ 16 mm) was then mixed with a Vortex mixer for various time intervals. After

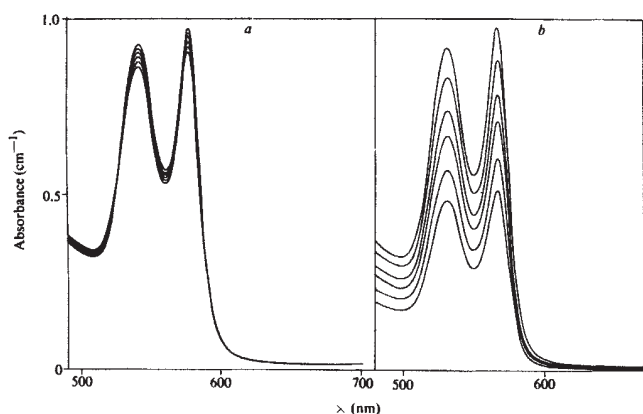


Fig. 1 Absorption spectra of the haemoglobin in supernatant fractions after mechanical treatment. Solutions of haemoglobin A (a) and S (b) in 0.1 M bis-Tris buffer, pH 7.0, were vigorously shaken by a Vortex mixer (Model K-500-4; medium position) for various time intervals (top to bottom 0, 2, 4, 6, 8, 10 min). The absorption spectra of the supernatants were measured with a Perkin-Elmer Coleman 124 Double Beam Spectrophotometer. The top curve in each diagram is the spectrum of untreated haemoglobin and the rest are those corresponding to the treated samples.

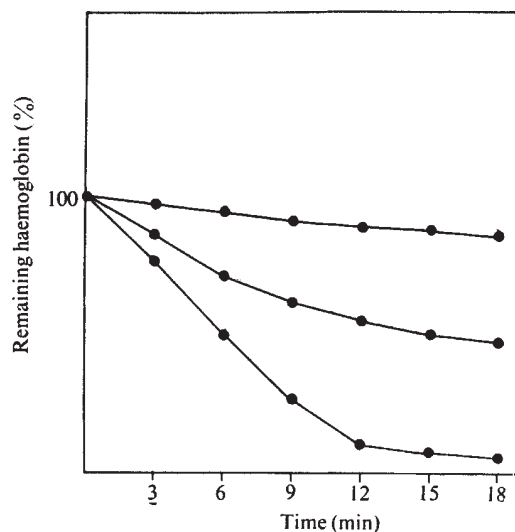


Fig. 2 Rate of denaturation of haemoglobin prepared from normal (A) (upper curve), heterozygous (AS) (middle curve) and homozygous blood (S) (lower curve). Haemoglobin solutions were shaken mechanically for different time intervals at 23° C. The haemoglobin in the supernatant was measured spectrophotometrically. 0.1 M Tris-HCl buffer, pH 8.

mixing, the solutions containing Hb S or haemoglobin from AS cells became turbid. The precipitates were removed by centrifugation at 6,000g for 5 min at 5° C. The absorption spectra of the haemoglobin remaining in the supernatant solutions after mixing for various time intervals are shown in Fig. 1. The results clearly show that oxyhaemoglobin S denatures at about a ten-fold faster rate than does oxyhaemoglobin A. Methaemoglobin was not formed in this experiment, as indicated by the flatness of the curves near 630 nm. Figure 2 compares the denaturation rates of haemoglobin from S and AS blood with that of Hb A. The rate of denaturation was calculated from the absorption change at 578 nm or 523 nm. The latter is the isosbestic point between oxy and methaemoglobin and was used when methaemoglobin was formed. Except in solutions below pH 5.0, no significant amount of methaemoglobin was formed by the mixing process. The initial denaturation rate of haemoglobin prepared from persons with sickle cell trait (AS) was approximately one-half of that prepared from patients with sickle cell disease (Hb S). After denaturation of approximately 50% of the total haemoglobin in the AS haemolysate the rate decreased to that of Hb A, suggesting that Hb S in the haemolysate denatured independently from Hb A. This result was examined further by densitometric quantitation of the haemoglobin bands separated by electrophoresis of the supernatant solution on cellulose acetate at pH 8.6. Before mixing, the electrophoresis showed 40% Hb S and 60% Hb A. After mixing for 10 min, the supernatant haemoglobin contained 10% Hb S and 90% Hb A.

The effect of pH on the rate of denaturation of Hb S is shown in Fig. 3. The denaturation rate was maximum at pH 8.5. Additional experiments demonstrated that the denaturation was also accelerated by increasing the ionic strength of the medium. Sixty per cent denaturation of Hb S occurred, however, after shaking for 10 min haemolysates prepared and diluted in water, indicating that salts were not essential. Denaturation was strongly temperature dependent, with Hb S being denatured more quickly at higher temperatures during mixing (Fig. 4). Haemolysates prepared from a patient with homozygous Hb C disease and from newborn infants (Hb A+Hb F) were as stable as those prepared from normal adults (Hb A).

Although the exact mechanism of the denaturation of

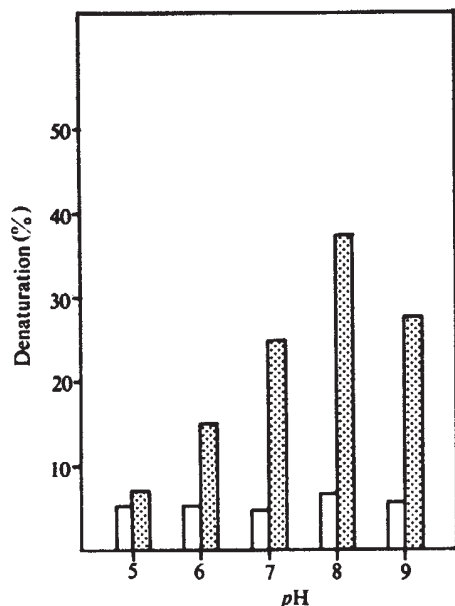


Fig. 3 Effect of pH on the mechanical instability of haemoglobin A (blank columns) and S (stippled columns). Denaturation (%) after 5 min shaking was compared at various pHs. 0.1 M PO_4^{2-} buffer.

Hb S is not known, the denaturation appears to involve an oxidative process, as the rate of precipitation of the fully oxygenated form was dependent on oxygen concentration. It was also found that the deoxy form of sickle cell haemoglobin is more stable than the oxy form. This difference in stability may be due to the inherent salt bridges present in the deoxy tetramer⁴, but it could also be related to a lack of oxygen.

As crystals of Hb A and Hb S have identical diffraction patterns⁵, the gross structures of the molecules are considered to be similar. The amino acid substitution at the sixth position of the β chain is external and therefore cannot be directly involved in the stability of the tetramer. This non-polar substitution may, however, shift a dynamic equilibrium between the folded and the unfolded forms of the monomer (or dimer) in favour of the unfolded form because of the interaction between non-polar side chains (M. F. Perutz, personal communication).

Although deoxygenation produces sickling of erythrocytes containing Hb S, denaturation of oxyhaemoglobin S may also cause increased red cell rigidity and change red cell shape. The presence of fully developed Heinz bodies (intracellular particles of precipitated haemoglobin) in

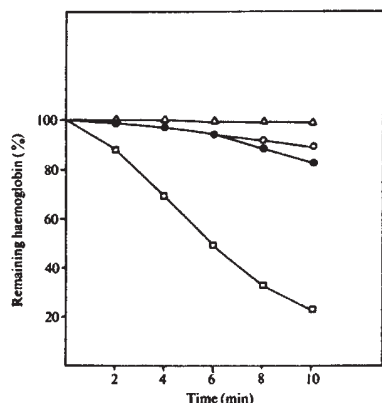


Fig. 4 Effect of temperature on the rate of denaturation of haemoglobin A and S. Δ , Hb A, 4°C; $\circ$, Hb A, 25°C; $\bullet$, Hb S, 4°C; $\square$, Hb S, 25°C. 0.1 M Tris-HCl buffer, pH 8.0.

untreated red cells from patients with sickle cell disease has been noted. These inclusions may be related to the denaturation process described here. Further examination of the mechanism of denaturation and the relationship of this denaturation to the properties of oxyhaemoglobin S in intact red cells is currently in progress in our laboratories.

We thank Professor M. F. Perutz for valuable comments.

This work was supported by grants from the US Public Health Service and State of Pennsylvania.

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Received February 13, 1973.

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The Regulation of Vitamin D Metabolism

VITAMIN D is hydroxylated in the liver to 25-hydroxycholecalciferol (25-HCC)¹, and this relatively inactive metabolite is further hydroxylated in the kidney to 1,25-dihydroxycholecalciferol (1,25-DHCC)² (Fig. 1), the most active form, or to 24,25-dihydroxycholecalciferol (24,25-DHCC)³, a less active metabolite. The proportion formed of these metabolites is markedly influenced by diet^{4,5}, but there is dispute about how this is achieved. One theory is that this involves parathyroid hormone and that this is the essential trophic factor which regulates the production of 1,25-DHCC⁶. There is strong evidence against this view⁷, however, and recent studies have cast further doubt: thyroparathyroidectomized rats can increase production of 1,25-DHCC under appropriate dietary stimulus⁸.

Fraser and Kodicek⁹, however, in apparent support of this role of parathyroid hormone, recently showed convincingly that removal of the parathyroid and ultimobranchial glands in chicks almost abolished the production of 1,25-DHCC. We have fully confirmed these results but show here that they were due to potentiation of an effect of vitamin D given to ensure post-operative survival. In different conditions removal of parathyroid and ultimobranchial glands from chicks has little or no influence on vitamin D metabolism.

When the parathyroid and ultimobranchial glands are removed from D-deficient chicks many of the birds die rapidly. A higher survival rate can be achieved if a large dose of vitamin D₃ is given 24 h before operation, the procedure adopted by Fraser and Kodicek⁹. Alternatives are either to ensure adequate intake of calcium post-operatively or to use less severely D-deficient chicks.

We have studied chicks in all three conditions. In our first experiment the parathyroid and ultimobranchial glands were removed from thirty chicks which had been fed a D-deficient

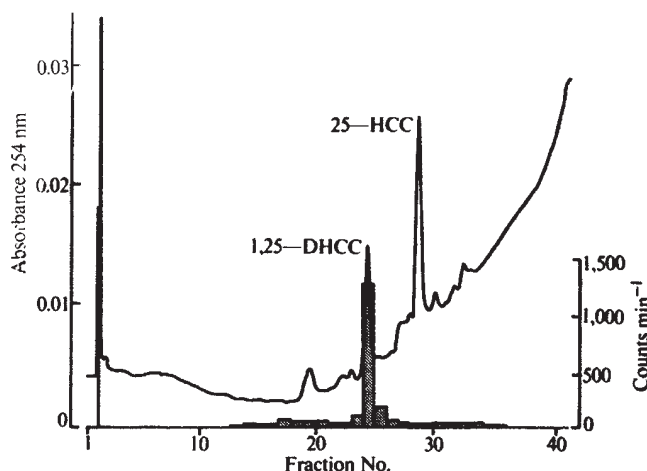


Fig. 1 The identification as 1,25-DHCC of a radioactivity peak from 'Sephadex' LH20 chromatography of a chick kidney homogenates. 75 ng synthetic 1,25-DHCC and 100 ng 25-HCC were added to a portion of the sample which was dissolved in methanol and injected into a 500 × 2.1 mm column (flow rate 1 ml min⁻¹; pressure drop 700 pounds per square inch) of ODS 'Permaphase' at 40° C on a Du Pont model 830 high-speed liquid chromatograph. Elution was performed with a linear gradient from water to methanol in which the methanol concentration increased by 5% (v/v) min⁻¹. The effluent was monitored at 254 nm and 30 s fractions were collected in a Gilson Microcol 80. The radioactivity in each sample was determined by liquid-scintillation counting and is shown as (stippled) columns against the ultraviolet trace after correction for dead volume. The radioactivity coincides exactly with the peak from added synthetic 1,25-DHCC.

diet for 3 weeks before operation (calcium 0.34%, phosphorus 0.54%). The chicks were allowed fat-free milk powder *ad libitum* post-operatively. Fourteen of the thirty survived and the remainder died in tetany. All fourteen sham-operated chicks survived operation. Table 1 shows that there was no change in the renal 1-hydroxylase activity for 20 h after operation.

These results seemed to be in direct conflict with those of Fraser and Kodicek and so a further series of experiments was carried out. Various D-deficient diets were used and in some experiments vitamin D was administered. In the others a reasonable survival rate was ensured by giving dried milk powder after operation or by calcium supplementation of the drinking water. Results are shown in Fig. 2. It is quite obvious that only the animals given vitamin D showed an effect of the operation, but as the groups were not all strictly comparable a confirmatory study was necessary. In this case we used chicks fed a D-deficient diet for 10 d only.

Table 1 Effect of Parathyroidectomy and Ultimobranchialectomy on 25-HCC-1-Hydroxylase Activity

Time after operation (h)	No. of birds	25-HCC-1-hydroxylase activity	Plasma (Ca) meq l ⁻¹
0 (sham)	14	49 ± 4.88	5.0 ± 0.09
1	1	46	3.2
3	3	38 ± 4.9	3.0 ± 0.12
6	2	23 ± 0.5	3.7 ± 0.65
9	2	42 ± 6.5	2.8 ± 0.20
20	6	42 ± 7.1	2.6 ± 0.18

Chicks were anaesthetized with ether and maintained under positive pressure ventilation with an endotracheal tube. The kidneys were removed and a 4% (w/v) homogenate prepared in Tris-acetate buffer at 4° C. The assay was completed¹⁰ using 25-HCC [26-(27)-methyl-³H]-25-hydroxycholecalciferol (13.1 Ci mmol⁻¹) from the Radiochemical Centre, Amersham. Metabolites were separated on 'Sephadex' LH20 (ref. 5).

The enzyme activity is expressed as the mean ³H % ± s.e. as 1,25-DHCC in kidney homogenates. Four replicate estimates were made from each bird. The plasma calcium is the mean ± s.e. of samples taken immediately before the birds were killed.

The results are unequivocal (Table 2, Fig. 3): vitamin D alone has a striking effect in depressing or abolishing 1-hydroxylase activity and in enhancing 24-hydroxylase activity; and removal of the parathyroid and ultimobranchial glands from chicks given vitamin D has a further marked effect in depressing 1-hydroxylase. The operation has no significant effect, however, in the absence of vitamin D. Clearly neither parathyroid hormone nor calcitonin is necessary for maintaining the enhanced 1-hydroxylase activity which is present in D-deficiency. Although Fraser and Kodicek⁹ did not observe an effect of D alone, this may have been because they used rather fewer numbers and because they did not estimate 24,25-DHCC.

The most likely hypothesis which reconciles all the published data is that a major determinant of the type of metabolite produced is the intracellular calcium concentration within some critical compartment in the renal cells⁵. There is experimental support for this view. Calcium ions have a powerful inhibitory effect on the production of 1,25-DHCC *in vitro*^{10,11}. Vitamin D is known to increase intracellular calcium¹² and this may be part of the explanation for its effect. Another factor may have been an increase in plasma phosphate, as this is also thought¹³ to inhibit the activity of the enzyme.

Both these effects of vitamin D would have been potentiated by removal of the parathyroid and ultimobranchial glands. Absence of calcitonin may have enhanced the increase in intracellular calcium¹⁴⁻¹⁶, and absence of parathyroid hor-

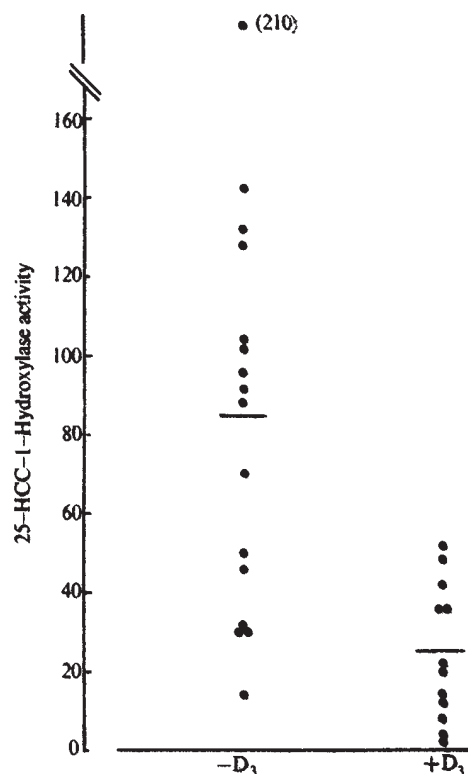


Fig. 2 25-HCC-1-hydroxylase activity after parathyroidectomy and ultimobranchialectomy (PTUX). Each value is the mean of two to four replicate estimations from one bird 14 h after operation as a percentage of sham-operated controls. Four D-deficient diets were given. The Ca and P % contents were: 0.34, 0.54; 0.68, 0.95; 1.0, 0.8; and 2.34, 0.54 respectively. Aluminium hydroxide (0.32%) was added to the 2.34% calcium diet to restrict phosphorus absorption. Results were similar in all diet groups. In one set of experiments cholecalciferol (2.5 µg) was given subcutaneously in ethanol 24 h before operation. The survival rates were: sham 25/25; D₃+PTUX 12/19; PTUX only 16/27. Only the D₃ treated birds showed a significant fall in enzyme activity after PTUX. The plasma calcium immediately before death in the operated groups as a percentage of the sham controls was: D₃+PTUX 68.4% ± 0.32, PTUX only 60.3% ± 0.42.

Table 2 Effect of Vitamin D₃ and of Parathyroidectomy and Ultimobranchialectomy (PTUX) on 25-HCC-1-Hydroxylase and 25-HCC-24-Hydroxylase Activity in Chick Kidney Homogenates

Operation	No. of birds surviving	Treatment	25-HCC-1-Hydroxylase activity	25-HCC-24-Hydroxylase activity	Plasma (Ca) meq l ⁻¹	Plasma (PO ₄) mg per 100 ml
Sham	4/4	-Ca -D ₃	14.39 ± 0.75	0.33 ± 0.08	4.6 ± 0.19	4.8 ± 0.11
	4/4	-Ca +D ₃	3.90 ± 0.85	5.11 ± 0.81	4.9 ± 0.19	7.2 ± 0.60
PTUX	4/4	-Ca +D ₃	0.41 ± 0.14	10.37 ± 0.66	3.1 ± 0.43	12.7 ± 2.60
	2/4	-Ca -D ₃	11.81 ± 1.78	0.40 ± 0.10	3.5 ± 0.45	6.6 ± 0.10
Sham	3/4	+Ca -D ₃	9.31 ± 1.20	0.22 ± 0.09	5.2 ± 0.32	4.7 ± 0.18
	4/4	+Ca +D ₃	0.95 ± 0.25	8.07 ± 0.87	5.3 ± 0.09	7.7 ± 0.84
PTUX	4/4	+Ca +D ₃	0.41 ± 0.13	10.68 ± 0.81	3.8 ± 0.42	6.2 ± 0.19
	4/4	+Ca -D ₃	7.68 ± 1.5	0.33 ± 0.13	4.0 ± 0.11	5.6 ± 0.38

Thirty-two chicks maintained for 10 d on a D-deficient diet (0.34% Ca; 0.54% P) were divided into two equal groups: one group (+D₃) received 2.5 µg cholecalciferol in 0.05 ml ethanol subcutaneously; the other (-D₃) received ethanol alone. After 24 h the groups were further subdivided into sham or operated (PTUX) subgroups of eight birds. After operation four of each subgroup (+Ca) were given 10 mg Ca h⁻¹ in 15% glucose and four (-Ca) water only. 14 h after operation blood was withdrawn by cardiac puncture for estimation of plasma calcium and inorganic phosphorus. The birds were then killed by decapitation and two or three replicate kidney homogenates prepared for enzyme assay. The enzyme activities are expressed as ng metabolite ± s.e. per 15 min produced from a homogenate of one quarter of a kidney. Vitamin D₃ has a significant effect ($P < 0.01$) in decreasing 1-hydroxylase and enhancing 24-hydroxylase activity. PTUX has no effect ($P < 0.05$) in the -D₃ group but has a marked effect ($P < 0.01$) in the +D₃ group.

more certainly increased the plasma phosphate to extreme levels in one group (Table 2).

It is important to emphasize that our results are not in conflict with suggestions that parathyroid hormone may increase the production of 1,25-DHCC in some circumstances⁹. But our experiments do show conclusively that parathyroid

hormone is not an essential trophic hormone for the production of 1,25-DHCC. Sadly, this seems to be another "beautiful and virtuous hypothesis butchered by a gang of ugly facts"¹⁷.

This work was supported in part by grants from the Medical Research Council and the Wellcome Trust. We thank Professor Derek Barton, Imperial College and Dr Maurice M. Pechet, Research Institute for Medicine and Chemistry, Cambridge, Massachusetts, for a gift of synthetic 1,25-DHCC, and Dr B. Mathieu de Fossey, Les Laboratoires Roussel, Paris, for the gift of 25-HCC.

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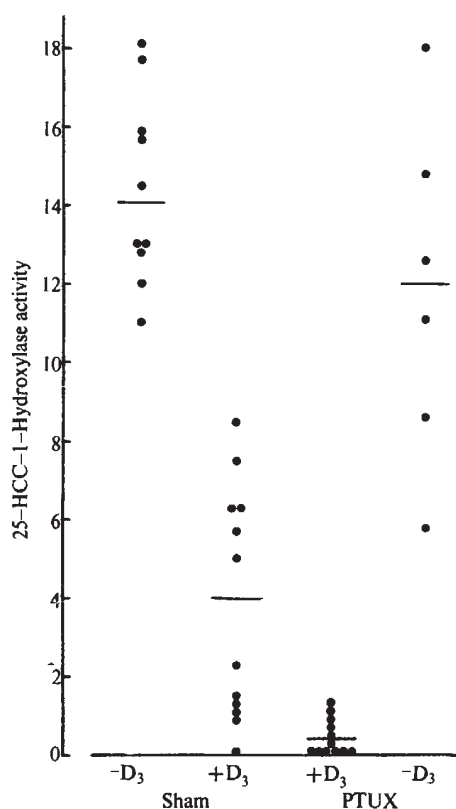


Fig. 3 The effect of cholecalciferol on 25-HCC-1-hydroxylase activity. Parathyroidectomy and ultimobranchialectomy (PTUX) or sham-operation was carried out 24 h after subcutaneous injection of 2.5 µg cholecalciferol in ethanol (+D₃) or ethanol alone (-D₃). The renal 25-HCC-1-hydroxylase activity was measured 14 h later without post-operative calcium supplementation (-Ca group, see Table 2). Vitamin D has a marked effect ($P < 0.01$) in both sham and PTUX groups: PTUX decreased enzyme activity only in the +D₃ group ($P < 0.01$). Each point represents the enzyme activity from one replicate expressed as ng 1,25-DHCC per 15 min from an homogenate of one quarter of a kidney.

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Tolerance of Sk-incompatible Skin Grafts

ALLOANTIGENS expressed on one cell type but not on another provide the basis for homograft reactions directed against particular tissues. The Sk alloantigens of mice are expressed on epidermal cells but not on haemopoietic cells¹. Thus C57Bl/6 (B6) mice that have been lethally irradiated and restored with (B6×A)F₁ spleen and bone marrow cells [abbreviated to (B6×A)-to-B6 chimaeras] invariably reject (B6×A) and A strain skin although they are permanent haemopoietic chimaeras. Rejection is accompanied by the production of antibody which is cytotoxic for A strain epidermal cells but not for A strain haemopoietic cells of any type². The antigen thus recognized, Sk.1, is absent from all tissues of B6 mice; B6 epidermal cells carry the alternative alloantigen Sk.2.

SJL and A skin. All H-2-incompatible skin grafts were rejected within a period little, if any, longer than that of H-2-incompatible skin grafts on normal mice. [Isolated Sk-incompatibility, represented by the A skin grafts on (B6×A)-to-B6 chimaeras (Table 1) gave rise to the usual somewhat protracted rejection of all grafts¹.] Thus maintenance of tolerance rather than immunosuppression is the likely cause of the chimaeras' delay in rejecting early skin grafts.

The use of much larger A strain skin grafts on (B6×A)-to-B6 chimaeras, intended to supply more Sk.1 antigen and so increase the degree of tolerance, did not give decisive results. So we next tried the effect of dispersed viable A strain epidermal cells² injected intravenously and intraperitoneally at the time of irradiation and restoration. As Table 2 shows, this achieved a high degree of tolerance; of fifteen (B6×A)-to-B6 chimaeras receiving these systemic inoculations of A strain epidermal cells,

Table 1 Prompt Rejection of H-2-incompatible Skin Grafts by Radiation Chimaeras*

Recipients (lethally irradiated)	Haemopoietic cell donor	Skin graft		Days after skin grafting											
		From	Incompatible for	0	10	20	30	50	60	100	200	300	350		
Number of mice with surviving skin grafts															
17 B6 mice†	B6	SJL	H-2	17	17	1	0								
		A	H-2	17	17	1	0								
11 B6 mice†	(B6 × A)	SJL	H-2	11	11	5	1	1	0						
		A	Sk only	11	11	11	11	11	11	7	2	1	0		

* 8×10^7 mixed spleen and bone marrow cells from B6 or (B6×A)F₁ donors were given intravenously within 24 h of lethal total body irradiation, as described previously¹. Donors and recipients were female mice about 3 months old.

† Each mouse received A and SJL tail-skin grafts side-by-side on one graft bed within 24 h of irradiation.

The ability of these chimaeras to reject A skin is ascribed to the fact that they lack Sk.1-bearing cells, assumed to be necessary for the maintenance of tolerance to Sk.1 antigen and so to A strain and (B6×A) skin grafts. An indication that this is so was implicit in the original work on Sk antigens. Thus rejection of donor skin grafts by chimaeras is considerably delayed if the grafts are made immediately after irradiation and haemopoietic restoration rather than some weeks later, suggesting that a skin graft provides sufficient Sk.1 antigen to maintain some degree of tolerance¹.

Table 2 Acceptance of Sk-incompatible (A) Skin Grafts by Lethally Irradiated B6 Mice restored with (B6×A) Haemopoietic Cells* and given Systemic Injections of Dispersed A Strain Epidermal Cells

Treatment of chimaeras	Days after grafting of A strain skin†					
	0	100	200	300	400	500
Mice with surviving skin grafts/mice grafted						
None	37/37	17/37	2/37	0/37		
A strain epidermal cells‡	21/21	21/21	19/20	19/20	13/17	10/15

* See footnote to Table 1.

† Skin was grafted within 24 h of irradiation.

‡ Trypsinized viable dispersed tail epidermal cells², 2.7×10^6 given intravenously and 2.7×10^6 given intraperitoneally, within 24 h of irradiation, followed by 2.5×10^7 given intraperitoneally on day 72.

Alternatively, the delay in rejection of early skin grafts could be attributed to immunosuppression caused by irradiation. But this explanation is rendered unlikely by the results in Table 1; B6 lethally irradiated mice were restored with either B6 or (B6×A) haemopoietic cells, and each then received grafts of

ten retained their A strain skin grafts for the entire period of observation (>500 d).

It was then necessary to show that the effect of the epidermal cells was specific for Sk.1. The experiment was repeated including a control group of twenty-four chimaeras receiving B6 (host-type) epidermal cells (Table 3); these produced no delay in the rejection of A skin grafts. On this occasion the degree of tolerance produced by A epidermal cells was not so marked (possibly because of a different schedule of inoculation) but six out of twenty-seven chimaeras of this group retained their A grafts throughout the observation period (>400 d).

As in the previous study¹, every chimaera rejecting donor skin was typed by haemagglutination and cytotoxicity tests for H-2, and none showed loss or decline of chimaerism, that is, their erythrocytes and lymph node cells gave H-2 reactions identical to the donor (B6×A).

Table 3 Sk.1 Specificity of the Tolerance produced by A Strain Epidermal Cells

Treatment of chimaeras*	Days after grafting of A strain skin†				
	0	100	200	300	400
Mice with surviving skin grafts/mice grafted					
None	7/7	4/7	1/7	0/7	
B6 epidermal cells‡ (control)	24/24	9/24	2/24	0/24	
A strain epidermal cells‡	27/27	25/27	19/27	10/27	6/27

* Of (B6×A)-to-B6 constitution; see footnote to Table 1.

† Skin was grafted within 24 h of irradiation.

‡ Trypsinized viable dispersed tail epidermal cells, 1.5×10^6 given intravenously and 3.5×10^6 given intraperitoneally, within 24 h of irradiation, followed by 1×10^7 given intraperitoneally on day 50, and 6×10^6 given intraperitoneally on day 85.

These results confirm our previous conclusions that these chimaeras reject donor skin because they are not immunologically tolerant to Sk.1 antigen, and that this is a result of the absence of Sk.1-bearing cells in the chimaeras. By supplying Sk.1 antigen, in the form of systemically inoculated epidermal cells, the rejection response to donor skin can be largely or entirely eliminated.

This work was supported in part by a grant from the National Cancer Institute.

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Localization and Release of Glutamic Acid in Relation to the Hippocampal Mossy Fibre Pathway

ABUNDANT clinical and experimental evidence indicates that the hippocampus is involved in temporal lobe epilepsy^{1,2}. Some of the symptoms of excitation associated with the disease may be related to the marked tendency of the hippocampus to produce seizures in response to mechanical, electrical and chemical stimuli³. Focal discharges in the temporal lobe could spread through direct afferents to the nearby entorhinal cortex⁴. Excitatory projections from entorhinal neurones form the final link in the chain between the sensory neocortex and the hippocampus by making synaptic contact with granule cells of the dentate gyrus. Axons arising from the granule cells synapse, in turn, on the apical dendrites of CA3-4 hippocampal pyramidal cells. These axons comprise the mossy fibre pathway⁵.

Repetitive stimulation of afferent fibres terminating on pyramidal cell apical dendrites is often associated with "spike complications" which develop after discharges, then ictal spikes³. Accumulation of a depolarizing substance in the vicinity of receptors on the tightly packed CA3-4 dendrites could establish an excitable focus with a low threshold for afferent stimuli. Certain acidic amino acids depolarize brain neurones^{6,7} and could, in some circumstances, alter the excitability of apical dendrites. Therefore, we determined the concentrations of free amino acids in the mossy fibre layer, and compared these concentrations with those in the apical dendrites of CA1 pyramidal cells which do not receive mossy fibres.

Whole brains were rapidly removed from cats asphyxiated with CO₂; frozen on the stage of a sliding microtome, and sectioned horizontally to expose the hippocampus. Tissue samples from the dendritic layers were dissected with the aid of a stereomicroscope and kept frozen until the amino acid concentrations were determined with an amino acid analyser. The most remarkable finding was the large concentration of free glutamine and glutamic acid in the mossy fibres of the CA3 area, relative to the concentrations in an analogous area of CA1 (Table 1). Five other amino acids (aspartate, threonine, serine, alanine, and glycine) were detected in the 5-12 mg samples, but were more uniformly distributed⁸. We also determined the relative enzymatic activity of L-glutamic acid dehydrogenase (EC 1.4.1.3) which catalyses the production of glutamic acid in cerebral tissue^{9,10}. The activity of the

enzyme in the mossy fibre layer was more than twice that in the CA1 apical dendrite layer. The presence of this zinc-metalloenzyme¹¹ in the mossy fibre pathway is noteworthy, as histochemical^{12,13}, autoradiographic^{14,15}, and ultrastructural studies¹³ have indicated that high concentrations of zinc are localized in large preterminal boutons synapsing on CA3 apical dendrites. Alternatively, the proposal¹⁶ that neurotransmitters may be stored as trace metal chelates may also be important in the mossy fibres, as glutamate forms ligand bonds with zinc¹⁷.

Table 1 Comparison of CA1 and CA3 Apical Dendritic Layers of Hippocampal Pyramidal Cells

	CA1	CA3
Free amino acids *† (nmol mg ⁻¹ protein)		
Aspartic acid (4)	15.3 ± 2.4	19.9 ± 0.6
Glutamine (3)	26.5 ± 2.3	41.5 ± 4.5
Glutamic acid (4)	44.6 ± 5.1	102.7 ± 6.4
L-Glutamic acid dehydrogenase *‡ (units mg ⁻¹ protein, n=3)	2.79 ± 0.34	6.54 ± 0.28
Zinc-dithizonate§ (histochemical reaction)	+	+++
⁶⁵ Zinc uptake in vivo (autoradiography)	△	△△△
Mossy fibre boutons¶	Absent	Present

* Results are expressed as means ± s.e.; numbers of cats in parentheses. Values for individual cats were obtained by averaging two or more analyses. Protein was measured by the Lowry method.

† Aspartic acid, glutamine and glutamic acid were assayed from tissue samples with a Beckman 120C amino acid analyser after extracting into 5% trichloroacetic acid; centrifuging at 23,500g for 30 min; lyophilizing the supernatant; and resuspending the residue in lithium-citrate buffer (pH 2.8). Aspartic values are not significantly different ($P > 0.05$). Glutamine and glutamic values are significantly different ($P < 0.05$, $P < 0.025$, respectively; two-tailed, two sample *t* test).

‡ A unit of enzyme activity represents 1 μmol of glutamic acid synthesized h⁻¹. Activity was measured kinetically by observing the rate of disappearance of NADH⁺ at 340 nm. Values significantly different ($P < 0.005$; two-tailed, two sample *t* test).

§ +, Faint colour; ++, intense colour (frozen sections after diphenylthiocarbazone, 100 mg kg⁻¹, intraperitoneal injection¹²).

|| △, Few positron tracks; △△△, multiple positron tracks¹³.

¶ By light and electron microscopic analyses⁵.

The relatively high concentration of glutamic acid in the mossy fibre layer prompted an investigation to determine if glutamate could be released by activation of the mossy fibres. In cats anaesthetized with methoxyflurane the overlying neocortex was aspirated and a collecting cup, positioned on the hippocampal surface, was continuously superfused with Ringer-Locke solution. A bipolar electrode within the cup recorded the surface electrical activity. This was displayed on an oscilloscope throughout the course of the experiments. Mossy fibres were activated by lowering a bipolar stimulating electrode into the entorhinal cortex until single stimulus pulses evoked wave-forms characteristic of pyramidal cell excitation⁵. Entorhinal stimulation significantly increased the rate of glutamate release (Fig. 1). An area immediately adjacent to the cup was also stimulated electrically as a test for specificity. Local stimulation elicited only a modest change in the release of glutamic acid (Fig. 1).

The presence of large amounts of glutamate in a circumscribed area of the hippocampus and the release of this excitatory amino acid in response to mossy fibre activation suggest that endogenous glutamate could initiate or reinforce the production of hippocampal ictal spikes. The relationship which may exist in the hippocampus between neuronal activity and glutamic acid is evident from reports that sustained release of L-glutamic acid from micropipettes in close proximity to pyramidal cells induces either a fast synchronized rhythm or seizure discharge¹⁸. Furthermore, the rate of glutamate release from experimental cortical foci increases dramatically during seizure activity¹⁹. Aberrations in glutamic acid meta-

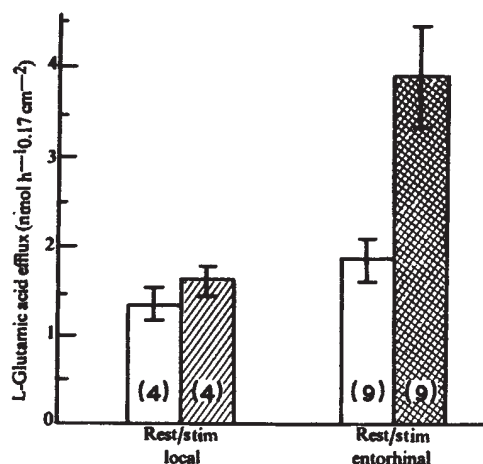


Fig. 1 Release of glutamic acid from cat hippocampus in the absence of stimulation (resting state) and in response to local and entorhinal electrical stimuli. Glutamate concentrations (nmol) in superfusates (cup area, 0.17 cm²; volume, 0.25 ml; Ringer-Locke solution, 13.6 μ l min⁻¹) were determined by a microenzymatic method²². Stimulus pulses (3 Hz, 0.5 ms duration, 10–40 V, for 1 h) were delivered to the entorhinal cortex or the hippocampal surface through bipolar electrodes (28 gauge stainless steel wire, 1.2 k Ω resistance, insulated except for the tips, <1 mm tip separation). The bars represent means ($\pm$ s.e.m.), number of animals in parentheses. Entorhinal stimulation significantly increased glutamate efflux when compared with release during rest ($P < 0.025$, paired t test) and during local stimulation ($P < 0.05$, two sample t test).

bolism in the brains of epileptics and of animal models of epilepsy are well known and a subject of current interest^{1,2,20–22}. Our results, linking glutamic acid with the mossy fibre pathway, should aid in the understanding of the interrelationships between cytological, physiological, and biochemical factors which may participate in the genesis of hippocampal seizures.

This research was supported by a grant from the US Public Health Service, National Institute of Neurological Diseases and Stroke.

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Received May 14; revised June 14, 1973.

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Pattern of Remyelination in the CNS

FOR more than a decade it has been known^{1–3} that glial cells are capable of forming new myelin sheaths around demyelinated axons in the central nervous system (CNS) but it is not known whether the new myelin is formed into segments bounded by true nodes and, if it is, whether the internodal length is appropriate to the axon diameter or inappropriately short as in remyelinated peripheral fibres. The different relationship between the myelin forming cell and axon in central and peripheral fibres makes this difficult to predict. Each Schwann cell in the peripheral nervous system (PNS) forms a single internode, whereas in the CNS a single oligodendrocyte usually forms a cluster of internodes on adjacent fibres^{4,5}. We have therefore studied the pattern of remyelination during recovery from acute experimental compression of the spinal cord, a lesion in which myelin destruction with preservation of axon continuity (demyelination) is followed by remyelination which commences in the third week⁶.

Observations were made on lesions from three adult cats and the findings compared with eight normal controls. In the experimental animals, a laminectomy was performed at L1 under pentobarbitone anaesthesia and the exposed posterior columns compressed through the intact dura for up to 3 h. Further details of the method of compression are given elsewhere⁶. The animals were killed under anaesthesia 21 d, 1 month and 3 months after compression, by retrograde perfusion through the abdominal aorta with 4 or 5% glutaraldehyde in phosphate buffer at pH 7.4 followed by Dalton's fluid⁷. The compressed segment of the cord was removed. Part of it was cut into thick (60 μ m) sections on a freezing microtome and teased under a dissecting microscope⁸. Measurements of diameter and internodal length of individual fibres were made using the method described by McDonald and Ohlrich⁹. The remainder was prepared for conventional light and electron microscopy.



Fig. 1 One-month lesion. An axon approximately 5 μ m in diameter is covered by an inappropriately thin myelin sheath. The arrows mark nodes (see text). The internodal distance is 67 μ m. Bar = 10 μ m.

Figure 1 shows a longitudinal section from a 1 month compression lesion in the posterior columns. An axon approximately 5 μ m in diameter is covered by an abnormally thin myelin sheath interrupted by two gaps (arrows). Examination of the gap regions under higher power showed that they had the characteristics of central nodes. On each side of a gap, the myelin lamellae terminated in cytoplasm-filled loops. Most of these were attached to the axolemma by transverse bands, the spacing between which was less regular than in normal animals. Some of the loops, although adjacent to the axolemma, were not attached to it. The internodal segment in Fig. 1 is 67 μ m long and was one of a series of four consecutive internodes ranging from 67 to 85 μ m in length. The myelin was uniformly thin in each. The minimum internodal length found in normal fibres of this diameter is 300 μ m⁹.

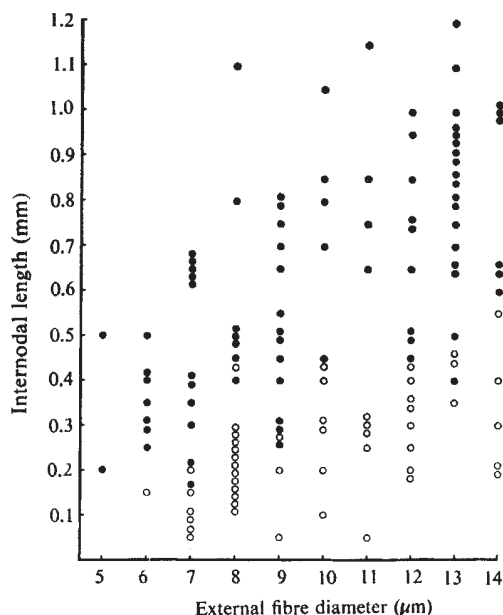


Fig. 2 Relationship between internodal length and external fibre diameter. (●), Fibres from normal animals; (○), fibres from compression lesions. (Details in text.)

(cf. Fig. 2). Although short internodes occur in the grey matter of the normal rat brain⁹, they are not found within the posterior columns of the cat spinal cord⁸. We conclude that these abnormally thin and short segments of myelin bounded by a node at each end are remyelinated internodes.

Further quantitative information about the dimensions of the remyelinated segments was obtained from light microscopic study of single fibres isolated by teasing from the peripheral parts of the lesion. Many fibres had short segments of thin myelin separated by nodal gaps from myelin of normal thickness. Measurements were made of fifty such fibres, and the internodal length of the thin segments compared with the internodal length of fibres of comparable diameter teased from three normal animals (Fig. 2). The diameter assigned to the fibre containing the thin segment was the mean of the diameters of the adjacent segments of the same fibre with myelin of normal thickness. As previously reported⁸, there was a direct relationship between internodal length and fibre diameter in the normal central fibres. Figure 2 shows that the majority of the thin segments have internodal lengths which are approximately 50% shorter than those found in control fibres of the same diameter. We conclude that during the first 3 months at least, newly formed internodes are inappropriately short for the fibre diameter. We are currently investigating acute compression lesions of longer duration to determine the extent to which the appropriate internodal length and myelin thickness are restored.

Other experiments have shown that Schwann cells of peripheral origin are capable of migrating into the spinal cord and forming myelin around central axons^{10,11}. The question therefore arises as to whether the short internodes in the present study were formed by glial or by Schwann cells. Two sets of observations show that the remyelination reported here was effected by glial cells. First, many of the remyelinated single fibres were isolated from animals in which careful light and electron microscopic examination of the remainder of the lesion failed to show Schwann cells. Second, on the rare occasions in one animal when remyelination by Schwann cells did occur, it was distinguished by a thick layer of cytoplasm in the paranodal region and a basement lamina which enclosed the whole internode. The thick cytoplasmic collar and basement lamina are absent from myelin sheaths of glial origin. The structure of the myelin forming processes resembles that of the oligodendrocyte, and was readily distinguished from

that of the fibril-containing astrocytes and the phagocytes⁶. We conclude that the thin internodes are formed by oligodendrocytes.

The extent to which remyelination occurs in the human CNS is still uncertain¹¹. Our demonstration of inappropriately short internodes may provide a new criterion for its identification. However, before interpreting the scanty direct¹² and indirect¹³ evidence for short internodes in multiple sclerosis as signs of remyelination, it is essential to have more information about the normal variation in length of internodes in different parts of the CNS in man, as in some species very short internodes are a feature of normal nerve fibres in the grey matter⁹.

Some recovery of function is characteristic of demyelinating lesions in the CNS. This raises the question of whether remyelination might restore transmission in fibres with conduction block due to demyelination¹⁴. Our study shows that the known morphological prerequisites for saltatory conduction are present in the remyelinated fibres. An examination of the physiological properties of the remyelinated fibres, and determination of the factors which govern the occurrence and completeness of remyelination in different types of central lesion, remain to be carried out.

We thank Drs D. N. Landon and R. G. Willison for helpful discussion, and Mr H. Long for preparation of the photographs. The work was supported by a grant from the Medical Research Council.

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Received April 24; revised June 15, 1973.

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Human x Mouse Somatic Cell Hybrid Clone secreting Immunoglobulins of both Parental Types

HYBRIDS between two distinct differentiated cell types usually do not express the differentiated characteristics of either parental cell. The synthesis of enzymes necessary for the metabolism of the cell continues, while the synthesis of specialized products of differentiation often is suppressed¹. Mouse myeloma cells, after fusion with mouse fibroblasts, follow this pattern; that is, the hybrid cells cease synthesis of immunoglobulin^{2,3}. A myeloma x lymphoma hybrid, however, continues secretion of immunoglobulin^{4,5}. We report

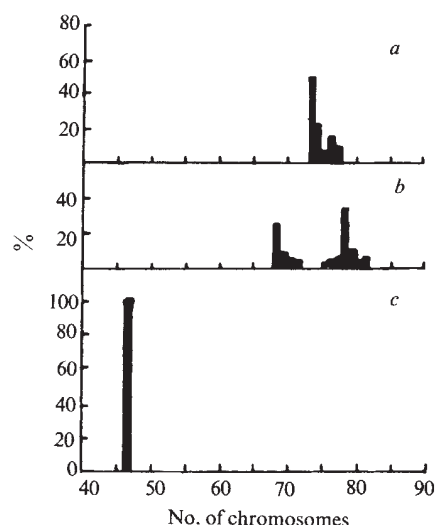


Fig. 1 Normal histogram showing the distribution of chromosomes from (c) human peripheral blood lymphocytes, (b) TEPC-15 mouse myeloma cells, and (a) clone H1C11 hybrid cells.

here the isolation of a hybrid clone, resulting from fusion of mouse myeloma cells secreting immunoglobulin of known specificity and human peripheral blood lymphocytes not secreting detectable immunoglobulin. The hybrid cells continue secretion of mouse immunoglobulin and initiate synthesis and secretion of human immunoglobulin.

TEPC-15 myeloma cells from Balb/c strain mice grow slowly *in vitro* in Eagle's minimum essential medium supplemented with 20% foetal calf serum and 150 mM L-glutamine (complete MEM). The cells remain viable in culture for 4–6 weeks, adhering to the bottom of plastic flasks. They continue synthesizing mouse IgA with antibody activity for *Pneumococcus C* polysaccharide⁶. Normal human peripheral blood lymphocytes remain viable *in vitro* for 4–5 d, after which rapid disintegration of the cells occurs spontaneously.

TEPC-15 myeloma cells and normal human peripheral blood lymphocytes were fused with the aid of inactivated Sendai virus. Two-week-old "cultures" of TEPC-15 cells were used as one parent. One thousand to five thousand TEPC-15 cells were incubated in a 30 ml Falcon flask at 4° C with 400 haemagglutinating units of β -propiolactone-inactivated Sendai virus (grown and inactivated according to the methods described by Klebe *et al.*⁷). After the virus was added, 2×10^6 purified human lymphocytes were added to the cultures. The lymphocytes were obtained by sterile venipuncture from a healthy donor (J.S.), separated from erythrocytes by gravity sedimentation for 3–4 h, followed by incubation for 1 h in a plastic Petri dish, to eliminate the adherent granulocytes. The lymphocyte parental population was 99% pure by morphological criteria. The cultures were incubated at 4° C for 20 min. They were then incubated at 37° C for another 30 min, after which warm complete MEM was added. The cultures were placed in an incubator at 37° C and gassed continuously with 5% CO₂ and 95% air. The medium was decanted completely every 4–5 d thereafter. All human parent cells disappeared from the cultures within 2 weeks. Many large cells could be seen at this time. Six to eight weeks after fusion, when discrete colonies could be seen, the cells were replated. After a further 4 weeks, clones were isolated. Three months after fusion (4 weeks after isolation) clone H1C11 grew in both mixed monolayer and suspension culture, and doubled every 35 h.

Metaphase chromosome spreads were prepared from this clone approximately 5 weeks after isolation. Vinblastine was used to arrest the cells in metaphase (0.075 M KCl hypotonic solution); fixation was in 1:3 acetic acid:

methanol, and the preparation was air dried on microscope slides^{8,9}. Analysis of human parental cell chromosomes prepared by this method showed a normal human chromosome constitution of 46 XY. TEPC-15 cells had a bimodal distribution of chromosomes, with clustering at sixty-eight and seventy-eight telocentric chromosomes (Fig. 1). The spreads with seventy-eight chromosomes had one very long telocentric chromosome with a secondary constriction (Fig. 2a). The human chromosomes, with the exception of the D and G groups, were distinguished readily from all the TEPC-15 chromosomes. Metaphase spreads from clone H1C11 revealed a modal number of seventy-three chromosomes (Figs 1 and 2), including two marker metacentric chromosomes characteristic of the human C and E groups (Fig. 2b). The very long myeloma marker telocentric chromosome was not present in any of the hybrid metaphase spreads examined. Eighty per cent of the spreads contained these two human metacentric markers, another 16% contained one and in only 4% of the chromosome spreads could no human marker chromosome be found.

The hybrid cell line continued producing myeloma protein with antibody activity as detected by agar gel diffusion. Precipitin lines formed in 1.0% agar between wells containing culture medium concentrated ten-fold and specific antiserum prepared in rabbits against mouse Ig, in which myeloma

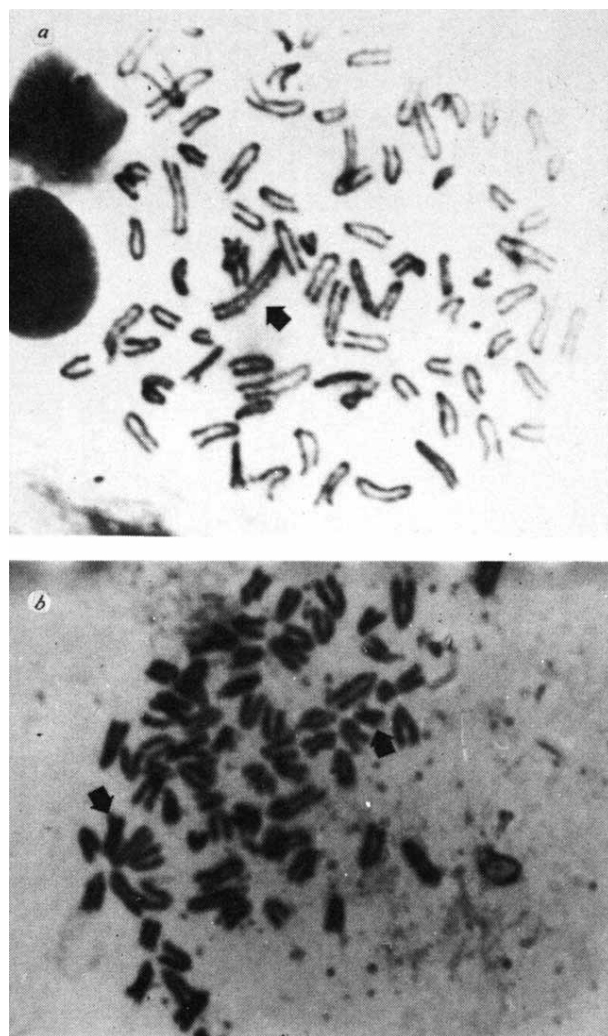


Fig. 2 a, Metaphase chromosome spread of a TEPC-15 mouse myeloma with eighty chromosomes. Note the very long telocentric marker. b, Metaphase chromosome spread of a clone H1C11 hybrid cell. Note the two human marker metacentric chromosomes (arrows) ($\times 550$).

protein served as an antigen. Precipitin lines also formed between wells containing ten-fold concentrated medium from the hybrid culture and *Pneumococcus C* polysaccharide, indicating that the Ig secreted by the hybrid retained its antibody activity.

To examine the hybrid cell cultures for the synthesis and secretion of human and mouse Igs, culture medium supernatant from hybrid cells incubated for 16 h with 30 μ Ci 3 H-leucine was precipitated directly with either rabbit antiserum to mouse Ig or rabbit antiserum to human Ig, and electrophoresed on SDS-containing acrylamide gels¹⁰. Immunoprecipitates formed after the hybrid cell culture supernatant was incubated with antimouse Ig contained optical density and radioactivity peaks co-migrating with heavy chain dimer (H_2) and light chain dimer (L_2), and an optical density peak co-migrating with precipitating γ globulin (H_2L_2) (Fig. 3). Hybrid cell culture medium precipitated with rabbit anti-human Ig showed three distinct peaks co-migrating with H_2L_2 , H_2 and L_2 . There was a shoulder on the H_2L_2 peak which, in addition to the H_2 and L_2 peaks, is associated with peaks of radioactivity (Fig. 3).

To determine whether individual hybrid cells synthesized both human and mouse Igs, clone H1C11 was examined by the fluorescent antibody technique. Fixed hybrid cell preparations were stained with fluorescein-conjugated rabbit

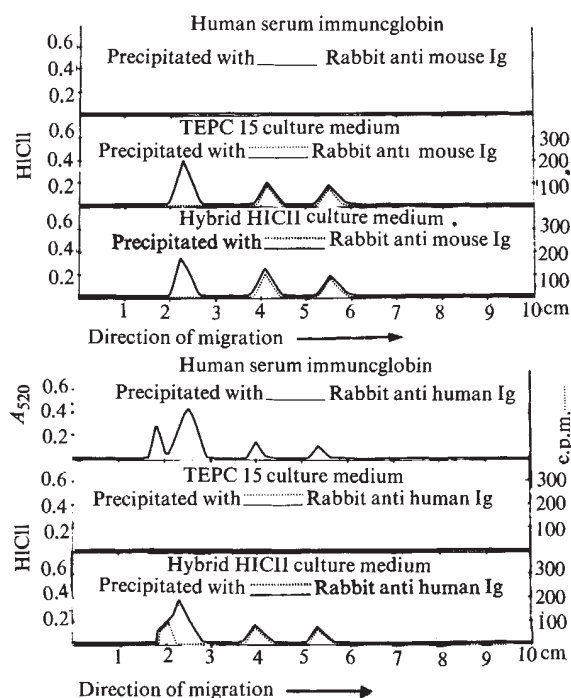
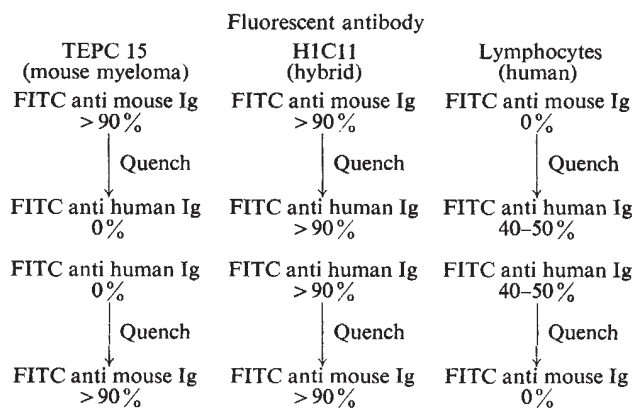


Fig. 3 Patterns of migration of human, mouse and hybrid clone H1C11 secreted Ig. Human serum was purified by $(NH_4)_2SO_4$ precipitation; the Ig was then directly precipitated with rabbit antiserum to human Ig. Hybrid cells and TEPC-15 C3 (culture-adapted myeloma cells) were incubated with 30 μ Ci 3 H-leucine in normal culture medium (excess cold leucine present) for 16 h. The cells were pelleted and the supernatant precipitated with rabbit antiserum to human Ig or rabbit antiserum to mouse Ig. The precipitates (human, mouse, hybrid) were redissolved in 0.1% SDS, and electrophoresed on 10 cm, SDS-containing, 5.0% acrylamide gels for 7 h (at 50 V). The gels were then stained with Coomassie blue, destained and scanned in a Gilford spectrophotometer with linear transport mechanism. The gels containing myeloma and hybrid medium were sliced (thirty slices per gel) and counted in a liquid scintillation counter. Bovine serum albumin added to hybrid culture medium and then precipitated with anti-BSA, yielded optical density bands corresponding to precipitating antibody (IgG) and BSA (68,000 molecular weight) but no radioactivity above background was detectable. Albumin (arrow 1) and haemoglobin (arrow 2) were used as markers for molecular size estimation.



Figures represent percentage of cells staining with fluorescent antiserum.

Fig. 4 Design of fluorescent antibody experiments. Both antisera were prepared by immunization of rabbits, $(NH_4)_2SO_4$ precipitation, and conjugation with fluorescein isothiocyanate (FITC).

antiserum to human Ig. Fluorescent cells were identified and the fluorescence was then quenched with strong ultraviolet irradiation. The cells were then stained with fluorescein-conjugated rabbit antiserum to mouse Ig (Fig. 4). The results indicate that most, if not all, of the cells synthesized both human and mouse Ig. The same experiment, done in the reverse order, yielded essentially the same results. The pattern of staining with antiserum to both human and mouse Ig was similar to the pattern of staining found with large lymphocytes and plasma cells¹¹. Staining with fluorescein-conjugated antiserum to mouse Ig could be blocked by previous addition of excess unconjugated antiserum to mouse Ig or with excess exogenous mouse Ig but not by antihuman Ig or exogenous human Ig. Staining with fluorescein-conjugated antiserum to human Ig could be blocked by antihuman Ig or human Ig but not by antimouse Ig or mouse Ig, showing the specificity of the staining.

Clone H1C11 is a hybrid resulting from the fusion of one mouse myeloma cell with one human lymphocyte. Absence of the very long acrocentric marker mouse chromosome indicates that the myeloma cell parent contained sixty-eight acrocentric chromosomes. The presence of only two metacentric chromosomes is the result of preferential exclusion of human chromosomes^{12,13}. The possibility remains that one or more telocentric chromosomes in the hybrid may be of human origin.

This hybrid clone secretes both human and mouse Igs. We have found no evidence of exclusion of synthesis or of secretion of either Ig in the hybrid cells. Individual cells synthesize and secrete both human and mouse Igs. The three radioactivity peaks on SDS gels, of hybrid medium precipitated with antiserum to human Ig, co-migrating with H_2L_2 , H_2 and L_2 , indicate either that some of the human Ig is IgA, in which H_2 and L_2 are non-covalently bound together, and/or that hybrid antibody is synthesized by these cells.

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Received February 28; revised June 4, 1973.

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Transplantation of Isolated Pancreatic Islets into the Portal Vein of Diabetic Rats

RECENT work from this laboratory indicates that intraperitoneal transplantation of isolated pancreatic islets ameliorates the effects of experimental diabetes in rats¹. Although the intraperitoneal siting of these islets reduces the hyperglycaemia and polyuria of streptozotocin induced diabetes in these animals, consistently normal values for blood sugar and urine volume are rarely achieved.

In normal circumstances, insulin from the pancreatic β -cells is secreted directly into the portal venous system and we have investigated the possibility that this intraportal site may provide

a more physiological environment for the transplanted islets and possibly more effective use of secreted insulin.

Pancreatic islets were collected from Lewis rats by the collagenase digestion method of Lacy and Kostianovsky² and separated from unwanted acinar debris by differential layering of dialysed 'Ficoll'³. This technique involves the mixing of digested pancreas with 'Ficoll' 25% (w/v) and the careful layering above this mixture of 'Ficoll' in decreasing concentrations (23%, 20% and 11%). Centrifugation for 10 min at 800g leaves the acinar tissue in the 25% 'Ficoll' while islets rise through the density gradient to settle at the 20–11% interface. The isolated islets (400–600) were then injected through a 1 ml siliconized syringe and No. 23 needle into the portal vein of diabetic Lewis rats of the same inbred strain as the donors. Diabetes had been induced in these recipient rats 3 weeks before by the intravenous injection of streptozotocin (65 mg kg⁻¹) and the study included a group of ten diabetic rats as control animals.

After the transplantation operation all rats were returned to their metabolic cages and observed for 4 weeks during which a free diet of rat chow and water was allowed. Urine volumes were measured daily and blood glucose levels estimated on alternate days.

The ten control animals were grossly diabetic with blood glucose levels varying between 270 and 720 mg/100 ml and urine volumes between 80 and 150 ml daily. Direct injection of pancreatic islets into the portal vein resulted in normoglycaemia and normal urine volumes in the five rats studied (Fig. 1).

These results suggest that if transplantation of isolated pancreatic islets is to be used in the treatment of clinical diabetes the portal venous system may be the site of choice for maximal effectiveness.

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Received April 11, 1973.

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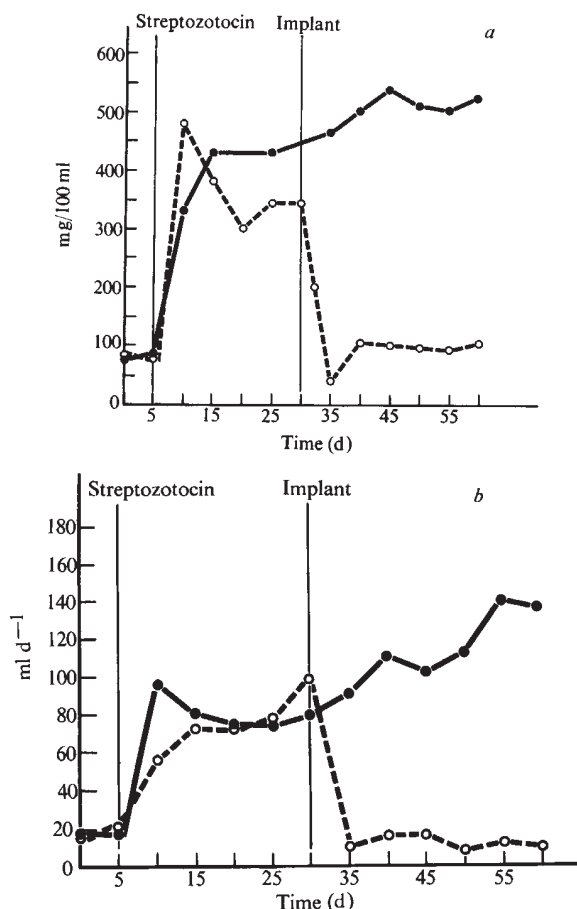


Fig. 1 Effect of portal vein implantation of pancreatic islets on the blood glucose (a) and urine (b) volumes of diabetic rats. (Mean of ten diabetic controls and five portal vein implants.) Values after transplantation: diabetic controls (●) blood sugar 270 to 720 mg%; urine volumes 80 to 150 ml daily; portal vein implant (○) blood glucose 66 to 140 mg%; urine volume 6 to 20 ml daily.

Sulphate Conjugation and L-Dopa Treatment of Parkinsonian Patients

CONJUGATION of catecholamines in man was first described for adrenaline by Richter in 1940¹. He suggested that the conjugated product was an ester of sulphuric acid. Richter and MacIntosh² later demonstrated that conjugation of adrenaline markedly reduced its pressor properties and proposed that conjugation is a mechanism of inactivation of the biological properties of this amine. Holtz and Credner³ administered L-dopa to several animals including man and isolated both free and conjugated dopamine (DA) from urine. Conjugation of two metabolites of DA, homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC), was observed by Shaw, McMillan and Armstrong⁴ when they isolated these two phenolic acids from the urine of human subjects who had received L-dopa orally. The conjugates of these compounds can be isolated by anion exchange chromatography^{5,6}. When conjugated derivatives in the eluates from anion exchange columns were hydrolysed by sulphuric acid^{5,6} or sulphatase⁵, the unconjugated parent compounds were identified by paper chromatography.

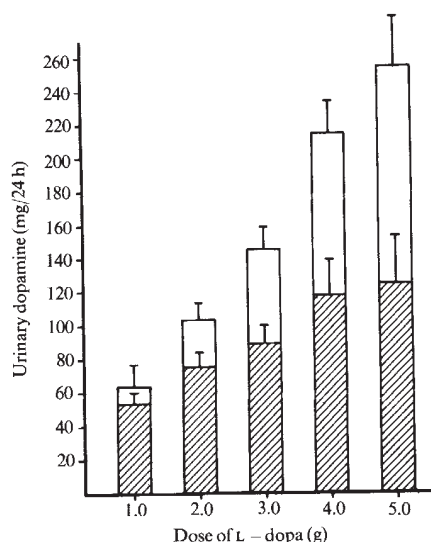


Fig. 1 Urinary excretion of DA from Parkinsonian patients treated with increasing dosages of L-dopa. Each value represents the mean $\pm$ s.e.m. of conjugated (▨) and nonconjugated (□) DA excreted (mg per 24 h).

Richter¹ suggested that sulphate conjugation might be limited with large amounts of phenolic substrate, and studies in rabbits indicate that conjugation of phenolic compounds with sulphuric acid is reduced as the dose of the phenolic substrate is increased^{7,8}. Because of the large doses of L-dopa employed in the treatment of Parkinsonian patients^{9,10}, it is possible that sulphate conjugation of DA and its metabolites will be reduced as the dosage of L-dopa is increased. An enhancement of the therapeutic effect of L-dopa with increasing dosages may be due to a reduction of conjugation with a consequent elevation of free DA. It is the free DA which is presumably responsible for many of the therapeutic and toxic effects of the drug. The purpose of this investigation was to study sulphate conjugation of DA and metabolites of DA with increasing dosages of L-dopa in patients with Parkinsonism.

Eight women and seven men with Parkinsonism were withdrawn from previous medication for at least a week before the study. The patients were aged from forty-nine to eighty-three with a mean age of sixty-seven. The duration of the disease in the twelve patients with primary Parkinsonism ranged from 3 to 13 yr (a mean duration of 7 yr) and the disease had been evident in the three patients with postencephalitic Parkinsonism for 8, 35 and 36 yr. Seven patients had severe and disabling disease (stages IV and V). Eight patients had mild to moderate disease and were still independent in their activities of daily living (stages II and III). The patients were admitted to a clinical research ward, and at least one 24 h urine sample was obtained before beginning therapy with L-dopa. The diet was not controlled except that foods containing catecholamines were restricted^{11,12}. L-Dopa treatment was started as 1.0 g d⁻¹ in four divided doses. The dosage was increased by 0.5 g d⁻¹ every 2 d to a dose of 3.0 g d⁻¹. The patients were discharged from the hospital and the dosage was increased by 0.5 g d⁻¹ every 1 to 2 weeks to a total daily dosage of 3.0 g d⁻¹ in two patients, 4.0 g d⁻¹ in three patients, and 5.0 g d⁻¹ in ten patients. Twenty-four hour urine samples were collected at the end of each dosage interval in bottles which contained 2.0 g sodium metabisulphite as a preservative. An aliquot of the urine was hydrolysed by boiling with hydrochloric or sulphuric acid¹³. Some samples were also hydrolysed enzymatically with glusulase as described by Smith and Weil-Malherbe¹⁴. No differences in the values of metabolites could be detected following the different methods of hydrolysis. Dopa, DA and DOPAC were isolated from urine of treated patients by alumina chromatography¹⁵ and were separated and assayed by paper chromatography according to the method of Sourkes¹⁶. DOPAC from the urine of untreated patients was also determined by this procedure. To attain the

greater specificity and sensitivity required for assay of DA, the method of Anton and Sayre¹⁵ was employed for urine from untreated patients. Dopa could not be detected consistently in the urine of untreated patients. HVA was determined by a modification of the procedure of Sato¹⁷. This method involves the isolation of HVA on 'Dowex' 1, an anion exchange resin, and elution with 18 ml of 2 N HCl. HVA is converted to a fluorescent product by oxidation with potassium ferricyanide. The urinary content of each metabolite was corrected for recovery. The recoveries were (mean $\pm$ s.e.m.): dopa, 72.1 $\pm$ 4.0%; DA, 76.0 $\pm$ 4.0%; DOPAC, 54.6 $\pm$ 2.7%; and HVA, 74.0 $\pm$ 4.7%. When urine samples were to be analysed for sulphate, hydrolysis was attained by boiling the urine with hydrochloric acid as described by Hawk, Oser and Summerson¹⁸. The glusulase method of hydrolysis¹⁴ was also used in a few experiments and similar results were obtained. Sulphate was determined in hydrolysed and nonhydrolysed urine samples by the turbidimetric procedure of Berglund and Sörbo¹⁹.

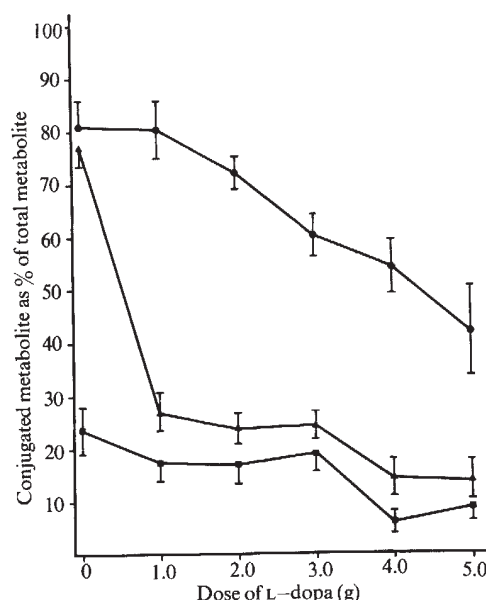


Fig. 2 Conjugation of urinary DA (●), DOPAC (▲) and HVA (■) with increasing dosages of L-dopa. The values represent the mean $\pm$ s.e.m. of % conjugation of the metabolite.

The excretion of DA in urine of untreated Parkinsonian patients amounted to 94 $\pm$ 21 (s.e.m.) μ g per 24 h. The amount of DA found in the urine of these patients increased to 659 $\pm$ 93 μ g per 24 h after the hydrolysis procedure. Thus, approximately 80% of the total DA was conjugated. When 1.0 g of L-dopa was administered, the excretion of DA increased markedly so that a total of 61.6 $\pm$ 7.6 mg per 24 h was excreted from hydrolysed urine (Fig. 1). The proportion of the total conjugated DA did not differ from that observed in the untreated patients. As the dosage of L-dopa was increased, the total DA excreted in the urine increased proportionately and the free DA represented a progressively greater fraction of the total. This can also be seen in Fig. 2 where the conjugation of DA is compared to the conjugation of DOPAC and HVA with increasing dosages of L-dopa. DOPAC excretion in the absence of L-dopa treatment was 321 $\pm$ 51 μ g per 24 h in nonhydrolysed urine and 1,683 $\pm$ 183 μ g per 24 h in hydrolysed urine. HVA excretion was 2,630 $\pm$ 310 μ g per 24 h in nonhydrolysed urine and 3,324 $\pm$ 366 μ g per 24 h in hydrolysed urine. After treatment with L-dopa, the excretion of total DOPAC and HVA (from hydrolysed urine) was proportional to the dose of dopa and represented 230 $\pm$ 10.6 mg DOPAC g⁻¹ of dopa administered per 24 h and 265 $\pm$ 12 mg HVA g⁻¹ of dopa administered per 24 h. The conjugation of

DOPAC and DA occurs to approximately the same extent but HVA is conjugated to a much smaller degree. After L-dopa administration, the % of DOPAC which was conjugated was reduced. This was observed with much smaller dosages of L-dopa than were required to decrease the proportion of conjugated DA. Conjugation of HVA was relatively unaffected until 4.0 to 5.0 g d⁻¹ of L-dopa was administered. As dopa was not consistently demonstrable in urine from untreated patients, the conjugation of endogenous dopa could not be estimated. Conjugation of dopa in three untreated patients was determined by administering 28.2 mg of ³H-L-dopa (200 μ Ci; 1.4 Ci mmol⁻¹) orally and measuring ³H-dopa in hydrolysed and nonhydrolysed urine samples. It was observed that 90.6 $\pm$ 4.2% of the ³H-dopa in the urine was conjugated. When the fifteen patients were treated with L-dopa, total dopa (from hydrolysed urine) was excreted in proportion to the dosage of L-dopa and represented about 5.5 mg g⁻¹ of L-dopa administered per 24 h. Of this amount, about 25% was conjugated at all dosages of L-dopa.

The metabolites of L-dopa are thought to be conjugated as sulphates and the organic sulphate excreted in the urine may be expected to increase. Indeed, both the organic sulphate and the proportion of total sulphate in the organic form was increased with increasing dosages of L-dopa (Fig. 3). The total sulphate (organic and inorganic) excreted was not markedly changed with increasing dosage of L-dopa and averaged 2.43 $\pm$ 0.14 g per 24 h. Glucuronic acid was also determined to obtain an index of glucuronide conjugation of L-dopa and its metabolites. Total glucuronic acid was determined colorimetrically by the method of Maughan, Evelyn and Browne²⁰. In untreated patients, total glucuronic acid excretion amounted to 357 $\pm$ 59 mg per 24 h. This was not changed upon administration of L-dopa. This is indirect evidence that glucuronic acid is not primarily involved in conjugation of metabolites of L-dopa as total glucuronic acid is known to increase when it is conjugated with large amounts of phenolic substrate²¹.

To confirm that the conjugation of DA was primarily with sulphuric acid, the sulphate conjugate of DA was isolated by anion exchange chromatography on 'Dowex' 1 according to the method of Goodall⁶. The material which was eluted where the DA sulphate would be expected was hydrolysed with hydrochloric acid and assayed for DA by the procedure of Anton and Sayre¹⁵. No DA was detected in this fraction before hydrolysis. The content of DA sulphate analysed in this manner corresponded well to the content of DA analysed in hydrolysed and nonhydrolysed urine without previous isolation on the 'Dowex' 1 column. The eluate which corresponded to the DA sulphate fraction of the 'Dowex' 1 column was also analysed by infrared spectrometry and the spectrum was identical to that of authentic DA sulphate.

The excretion of DA and its metabolites after therapy with L-dopa has been measured in numerous studies but little attention has been given to the sulphate conjugation of these substances. Cotzias *et al.*⁹ observed that the excretion of free DA was not proportional to the dosage of L-dopa, but rather that the % of L-dopa excreted as free DA increased linearly as the dosage of L-dopa was increased. Their results were similar to those of this study in that the proportion of free DA increased with increasing dosages of L-dopa. The data from this study indicate that this can be explained by a decrease in the % of conjugated DA with large dosages of L-dopa.

The decrease in the % of DA sulphate excreted could be the result of either increased sulphatase activity or decreased sulphate conjugation. There is evidence to indicate that the latter may be the more significant. Bray *et al.*²² demonstrated in the rabbit that the level of endogenous inorganic sulphate is normally rate limiting in the formation of arylsulphates *in vivo*. On the administration of such substances as phenol⁷ and adrenaline⁸ there is a dose-related decrease in the proportion of the conjugated product.

The source of endogenous sulphate seems to derive from the catabolism of protein rather than from the diet. Young and Maw²³ have shown that cystine is the major source of sulphate

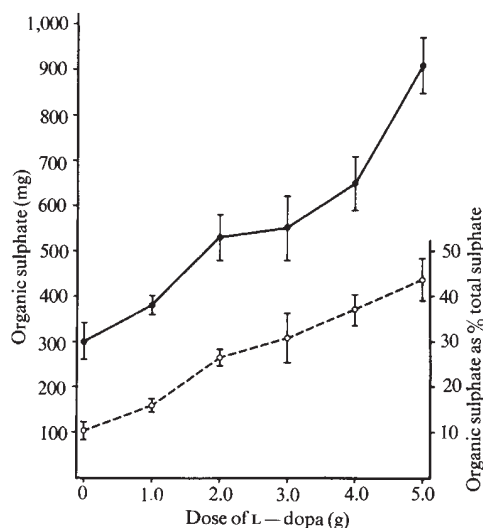


Fig. 3 Urinary excretion of organic sulphate with increasing dosages of L-dopa. Values are expressed as either mg of organic sulphate per 24 h $\pm$ s.e.m. (sulphate in hydrolysed urine—sulphate in nonhydrolysed urine) (●—●) or % organic sulphate $\pm$ s.e.m. (○—○).

used in ethereal sulphate synthesis. It is clear that the total sulphate cannot be rate limiting as the rate of sulphate conjugation is markedly reduced with 5 g of L-dopa and yet only 45% of the total sulphate in the urine is present as organic sulphate. Thus, it is probable that the pool of sulphate available for conjugation does not freely equilibrate with total body sulphate and it is the sulphate pool available for conjugation which becomes rate limiting.

Preliminary evidence from this laboratory on the metabolism of ³H-L-dopa given orally and intravenously suggests that conjugation of dopa and its metabolites takes place predominantly in the intestine. Other studies have shown that adrenaline²⁴ and isoproterenol²⁵ are conjugated to a greater extent after oral than after parenteral administration. It is also known that the intestinal wall as well as the liver is capable of sulphate conjugation²⁶. Sulphate conjugation of phenolic biogenic amines and amine metabolites is also known to take place in the brain²⁷⁻²⁹. If suppression of conjugation occurs in the brain this would result in more free DA being available to exert a therapeutic or toxic effect. There is indirect evidence that changes in behaviour are related to free DA rather than to conjugated or total DA. Weil-Malherbe and Van Buren³¹ observed that free DA in the urine of Parkinsonian patients was markedly reduced when compared to controls, but that conjugated DA in Parkinsonian patients was not significantly different from that of healthy subjects. Similarly, Mattock *et al.*³⁰ observed that free DA was reduced in the urine of schizophrenic patients, but that conjugated DA was not significantly different from that of normal subjects. Preliminary analyses of the patients of this study suggest that the rigidity ($P < 0.005$) and akinesia ($P < 0.010$) in the untreated patients correlate negatively with free DA in the urine but are not correlated with conjugated or total DA in the urine.

The results of this study suggest that high dosages of L-dopa reduce the rate of sulphate conjugation of DA allowing for marked increases in the free DA which may be available to produce its therapeutic as well as its toxic effects.

We thank Miss Amelia Marlowe and Mr Robert Arnold for technical assistance. We also thank Dr Robert Murphy for the assistance in identification of DA sulphate. The L-dopa (Larodopa) received by the patients was generously contributed by Hoffmann-La Roche, Nutley, New Jersey. This study was supported in part by a US Public Health Service grant and in part by a grant from the General Clinical Research Centers

Program of the Division of Research Resources, National Institutes of Health.

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Isolation of a Factor from Human Seminal Plasma that Promotes Fertilization

EXTENSIVE studies on the seminal plasma of man and other species have attempted to define the role of various components on the metabolism of spermatozoa, on the female reproductive tract and on the motility of spermatozoa and their capacity to fertilize the ovum¹⁻⁷. These studies have provided evidence of a role for fructose, cyclic AMP, prostaglandins and other constituents^{1,8}. We report here a newly discovered small molecular weight substance in human seminal plasma with a beneficial effect on the fertilization by epididymal sperm of ova of two mammalian species.

Seminal plasma of normal human males was pooled and centrifuged at 1,440g for 30 min. The supernatant was again centrifuged at 30,000g for 45 min. The clear seminal fluid

obtained was chromatographed on a 'Sephadex' G-15 column, and eluted with 20 mM ammonium bicarbonate (Fig. 1).

Of the five fractions, fraction III was collected from different runs, pooled and lyophilized. The residue was taken in distilled water and lyophilized. This process was repeated three or four times to remove the ammonium bicarbonate added. The solid residue was reconstituted in a small volume of distilled water. Further separation of fraction III was carried out by paper chromatography (see legend to Fig. 2). The paper was then sprayed with diazo reagent⁹, which gives evidence of primary, secondary or tertiary amino groups, as well as phenolic groups in the molecule. A compound staining light orange was obtained that is denoted as P (promoter) factor. It was eluted with water, freeze dried and stored at 4° C.

The effect of P factor was tested on fertilization in rabbits and mice. The action on *in vivo* fertilization in rabbits was assessed by the procedure described previously¹⁰. Sperm from the cauda epididymis of a male rabbit were collected in Krebs-Ringer phosphate buffer, pH 7.4, supplemented with 5 mg ml⁻¹ glucose and 5% heated (56° C, 30 min) rabbit serum (KRPGS). The sperm were capacitated by injection into the uteri of a virgin doe primed with 75 IU of HCG (Ayerst Lab). After 12 h the uteri were flushed with KRPGS. The suspension of capacitated sperm was diluted to 4 to 6 × 10⁵ sperm ml⁻¹ and was divided into two parts. P factor (250 µg ml⁻¹) was added to one and the other received an equivalent volume of the vehicle; both were incubated at 37° C for 20 to 30 min. Superovulation was induced in mature virgin does by injecting 170 IU of PMS (Sigma) subcutaneously, followed 72 h later by 150 IU of HCG intravenously. After 15 to 16 h of HCG administration, capacitated sperm with or without test substance were inseminated into the oviducts. One fallopian tube of each rabbit received 0.05 ml of treated sperm suspension and the other received 0.05 ml of untreated sperm suspension (control). The fallopian tubes were flushed with 0.9% saline 26 h later. The ova were collected and examined for fertilization; ovulation points were also counted on the ovaries. Figures 3a and b show the typical appearance of fertilized and unfertilized ova at this stage.

Table 1 Effect of P Factor on *in vivo* Fertilization of Rabbit Ova

Test substance	No. of oviducts examined	Ova fertilized/Total number of ova	Ova fertilized* (%)
None, control	20	95/190	50
250 µg ml ⁻¹ P factor	20	172/200	86

*P < 0.001.

Table 1 summarizes the results of the effect of P factor on fertilization of rabbit ova. Of 200 ova 86% were fertilized and

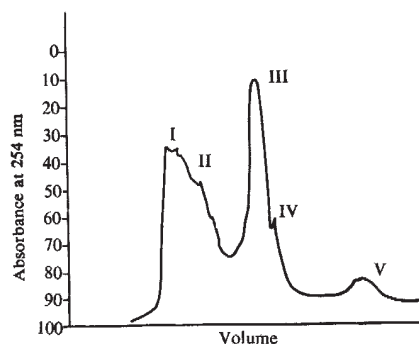


Fig. 1 Fractionation of human seminal plasma on 'Sephadex' G-15 column (3 × 80 cm), clear seminal fluid (10 ml) was loaded. Elution was carried out with 20 mM NH₄HCO₃. Flow rate was adjusted to 1.5 ml min⁻¹. The profile was recorded by an automatic LKB fraction collector, fitted with uv cord to monitor the absorbance continuously at 254 nm.

Table 2 Effect of P Factor on *in vitro* Fertilization of Mouse Ova

Test substance	Amount of P factor (μg)	Ova fertilized/Total number of ova	Ova fertilized (%)
Experiment I			
Control (0.5 ml sperm suspension)	—	1/33	3
+ P factor	40	15/27	55
	80	12/16	75
	120	24/30	80
	160	28/31	90
Experiment II			
Control (0.5 ml sperm suspension)	—	2/17	12
+ P factor	40	5/20	25
	80	12/23	50
	120	6/9	66
	160	14/15	93
Experiment III			
Control (0.5 ml sperm suspension)	—	8/18	44
+ P factor	40	12/24	50
	80	14/20	70
	120	19/23	83
	160	17/19	90

appeared to have undergone normal cleavage and development to the four- or eight-cell stage in the treated group compared with control oviducts in which of 190 ova shed 50% were fertilized.

Experiments were extended to another species to see whether P substance affected fertilization in ova other than from rabbits. The fertilization of mouse ova was studied *in vitro* using the procedure of Miyamoto and Chang¹¹, with slight modifications. Superovulation was induced in five mature Swiss albino mice by an intraperitoneal injection of 5 IU of PMS followed 44 to 48 h later by 5 IU of HCG. The mice were killed at 14 to 16 h after the injection of HCG. The oviducts were removed and placed under paraffin oil in a watch glass at 37° C. Eggs in the cumulus clot were released into the oil. The paraffin oil, sub-layered with a small volume of sterile saline, was equilibrated with 5% CO₂ in 95% air. The sperm suspension was prepared by mincing cauda epididymis of a mature Swiss albino mouse in a watch glass containing 5 ml of the medium¹². Aliquots of 0.5 ml of sperm suspensions (1 to 5 × 10⁶ sperm ml⁻¹) were incubated with 40, 80, 120 or 160 μg of P factor for 20 to 30 min at 37° C. Sperm suspensions with and without treatment were added to separate watch glasses containing the ova under paraffin oil. After incubation at 37° C for 5 to 6 h, the eggs were removed and washed in 2 ml of culture medium. They were then transferred to a culture medium of the same com-

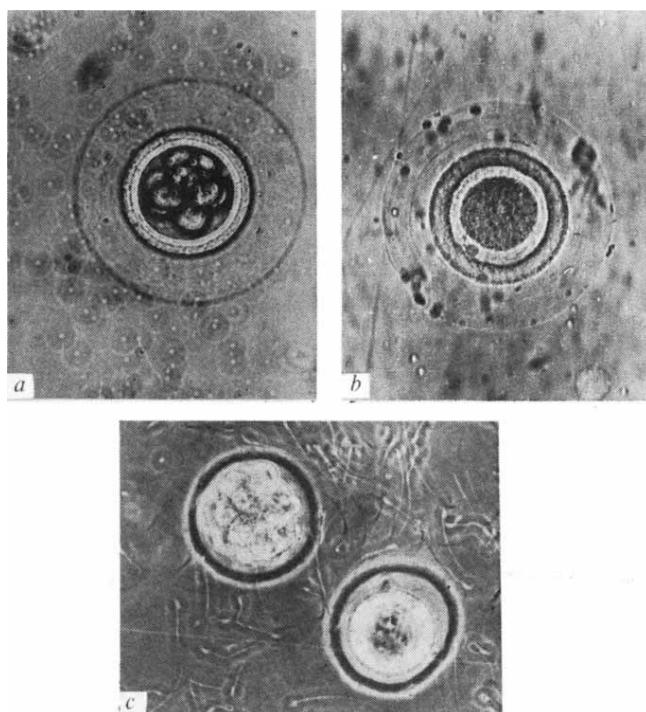


Fig. 3 a, Fertilized rabbit ovum at eight-cell stage; b, unfertilized rabbit ovum; c, mouse ovum fertilized *in vitro* at eight-cell stage; d, unfertilized mouse ovum.

position as that used by Whitten and Biggers¹³, except that the concentration of sodium pyruvate was 0.55 mM. The medium was layered with paraffin oil. After 24 h, the eggs were examined for fertilization and cleavage (Figs 3c and d).

The results are given in Table 2. The % ova fertilized varied considerably in the control group (3 to 44%). Treatment with P factor substantially increased the number of ova fertilized. Data further show that with the increase in concentration of P substance, the number of ova fertilized increased progressively such that 93% of eggs were fertilized in the presence of 160 μg /0.5 ml of P factor. This substance has thus a notable influence on the fertilization of ova from two mammalian species. Although the substance is of human origin, experimental limitations did not permit its testing in humans. It is not unlikely, however, that P factor has a role in promoting fertilization in human and other species.

This work has been supported by research grants of the WHO (Human Reproduction Division), The Ford Foundation, The Population Council Inc., New York, and a grant from the Office of Naval Research.

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Received April 3; revised May 1, 1973.

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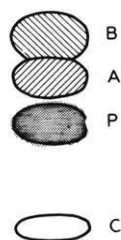


Fig. 2 Fraction III was further resolved by descending paper chromatography on Whatman Paper No. 1. The solvent system was *n*-propanol : 1 N acetic acid (3 : 1). Spots A, B and C show absorption in ultraviolet light. P is the "promoter factor", staining light orange with diazo reagent⁹.

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Zirga Virus, a New Arbovirus isolated from Bird-infesting Ticks

IN October 1968, the Department of Entomology at the London School of Hygiene and Tropical Medicine received from the Chief Medical Officer of the British Petroleum Company Ltd, two nymphs of an argasid tick. They were collected from the island of Zirqa (Zarrakkuh) (24°53'N, 53°04'E) off the coast of Abu Dhabi in the Persian Gulf and were associated with ground-nesting ospreys (*Pandion h. haliaetus*) and Socotra cormorants (*Phalacrocorax nigrogularis*) which appear on the island towards the end of August and remain till early February. The island is barren and uninhabited by humans. Abu Dhabi Marine Areas Limited (ADMA) have a radiomast and generator on the island and ADMA personnel make regular visits for routine maintenance of the plant. Local fishermen also visit the island from time to time. Attention was drawn to the presence of ticks when personnel were bitten by them and reacted with allergic symptoms, septic sores and high temperature. Case history notes are given by Hoogstraal, Oliver and Guirgis¹. Blood smears from a patient who was ill following tick bites were negative for spirochaetes, protozoa and microfilariae. The ticks were identified by one of us (M. G. R. V.) as *Ornithodoros* sp. and sent to Dr H. Hoogstraal of NAMRU-3, Cairo, who assigned them to *Ornithodoros (Alectorobius) muesebecki* described from specimens collected from a booby colony on Hasikiya Island, South Yemen Republic, in the Arabian Sea².

In November 1969, several specimens of this tick collected from the nests of cormorants on Zirqa Island by the late Dr R. M. Oliver, Senior Medical Officer of the British Petroleum Company, were received. Because it seemed possible that the symptoms in humans following tick bites had a viral aetiology, the ticks were processed for virus isolation.

Twenty-five nymphs of all stages were killed by chilling 2 d after receipt and ground up in 2 ml of 0.75% bovine albumin in borate buffered saline at pH 9 (BABS) with 1,000 U of penicillin and 1 mg of streptomycin ml⁻¹. After centrifugation, the supernatant fluid was inoculated into two litters of eight 1-d-old white mice (TO strain), each mouse receiving 0.02 ml intracerebrally and 0.03 ml subcutaneously. The rest of the tick suspension was stored in sealed glass ampoules at -60° C. One litter was lost through the mother's eating the babies. Eleven days after inoculation, two of the mice in the second litter were paralysed and three were ill. A 10% brain suspension in BABS from two of the ill mice was inoculated intracerebrally into a further four litters of 2-d-old mice. A broth culture of the brain suspension was negative. The isolate in the second passage in suckling mice had an incubation period of 6 d. Third passage mouse brain suspension of the prototype strain (A2070-1) had an intracerebral titre of 7.0 log per 0.02 ml in suckling mice. The agent was pathogenic to adult mice also and the incubation period in 3 to 4-week-old mice inoculated intracerebrally with 10⁻⁴ dilution of third passage suckling mouse brain suspension was 6 d. Third and fourth passage mouse brain suspensions titred 5.9 log to 6.5 log per 0.03 ml intracerebrally in adult mice. The agent has been named Zirqa virus. The virus has been re-isolated from the original tick suspension after more than 3 yr storage at -60° C and the identity of the isolate confirmed by complement fixation tests. Additional strains of the virus were also isolated from ticks sent to NAMRU-3, Cairo, by Dr R. E. Williams.

Further tests done at the Microbiological Research Estab-

lishment at Porton (D. I. H. S. and E. T. W. B.) showed that the mouse intracerebral LD₅₀ of the agent was reduced by 3.4 log after treatment with an equal volume of ether at 4° C for 1 h and by ≥4.6 log after treatment with a final concentration of 0.1% sodium deoxycholate at 37° C for 1 h. An antigen prepared from third passage mouse brain suspension after sucrose-acetone extraction, sonication and trypsin and protamine treatment and tested in haemagglutinin inhibition (HI) tests against antisera to selected arboviruses showed that the agent did not belong to arbovirus serological groups A, B, Bunyamwera, Simbu, California, Kaisodi or Bakau.

A screening complement fixation test done at the Yale Arbovirus Research Unit (J. C.) with immune serum for Zirqa virus and all antigens from non-group B tick-borne viruses to hand (about 40) established the virus as a new agent in the Hughes group of tick-borne arboviruses, closer to Farallon than to the other viruses of the group (Table 1).

Table 1 Relationship between Zirqa Virus and Other Members of the Hughes Group as Determined by Complement Fixation Tests

Antigen	Zirqa	Serum Farallon	Punta Salinas
Zirqa (A2070-1)	512/128*	64/64	1:8
Farallon	64/512	512/512	1:32
Hughes	16/16+		1:32
Soldado	16/16		1:8 traces
Punta Salinas (Cal Ar 888)			1:128
Normal	0/0	0/0	0

* Reciprocal of serum titre/reciprocal of antigen titre.

All the viruses in the Hughes group, including Zirqa, are associated with marine birds and *Ornithodoros* spp. The investigation of similar biocenoses elsewhere may reveal the existence of other viruses. There is no known human illness associated with Farallon, Hughes or Soldado viruses. Punta Salinas isolated from *Ornithodoros amblyus*, a species closely related to *O. muesebecki*, has been identified as a member of the Hughes group (H. Johnson, personal communication). Dr Johnson informs us that this virus and another, Huacho virus, belonging to the Kemorovo-Chenuda group, may be likely candidates for the febrile illness associated with tick bites that occurs among guano workers off the coast of Peru. On Zirqa Island, the ticks appeared within a matter of minutes on the nearby ground surface when a person placed a bare foot near cormorants' nests and some dexterity was required to avoid being bitten¹. There is no clear evidence so far that Zirqa virus was the aetiological agent of the illness among the petroleum industry workers on Zirqa Island, but the isolation of this virus underlines the potential danger to man when he makes effective contact with such silent foci of infection.

We are grateful to the late Dr R. M. Oliver and to Dr J. G. Clothier, Chief Medical Officer of the British Petroleum Company Ltd, for bringing our attention to the tick and for much of the information about the island and the tick.

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Form and Role of Deformation in Excitation of an Insect Mechanoreceptor

THE proprioceptive campaniform sensilla of insect legs are thought to sense strains developed in the cuticle of the leg in weight bearing and walking. Pringle¹ proposed that a ridge in the cuticular cap of the sensillum bulges outward under compressional shear stress, thus stretching and exciting the underlying single bipolar sensory neurone. His main assumption was that the mechanical compliance of the cuticle should differ in at least two subregions of the cap. This hypothesis has been widely accepted and is probably substantially correct, but several essential details have not been tested directly. Until now, the structure of the cap has not been shown clearly in cockroach legs, although it has been in dipterous halteres^{2,3}, and neither the mechanical properties of the cap cuticle nor the magnitude and direction

of the excitatory deformation have been measured, although the fine structure of the bipolar neurone^{4,5} and its responses to punctate stimulation^{6,7} are known.

We describe here the surface structure of these sensilla as revealed by the scanning electron microscope, and the mechanical compliance, surface deformation and sensitivity as measured by a new application of punctate mechanical stimulation. All work was done with the campaniform sensilla of Pringle's¹ tibial group six. Scanning electron micrographs were taken in the cockroach *Blaberus giganteus* and the physiological measurements in *B. discoidalis*. A preliminary report has been presented⁸.

Fig. 1A shows, at low magnification, the caps of fifteen sensilla of group six recessed a few micrometres into the surface of the tibia. A single cap comprises a directionally orientated convex oval dome located within a crater-like depression (seen at high magnification Fig. 1B). The dome, decorated by a tiny, centrally-located moulting pit^{9,10}, sits in a narrow groove which defines its perimeter, and is slung from the walls of the crater by a thin, curved field of exocuticle. A narrow, irregular depression, probably formed by the underlying enveloping cell, runs proximally from the edge of the dome to the edge of the cap. The proprioceptive campaniform sensilla of cockroach legs thus differ substantially from their counterparts on the halteres of flies^{2,3}. The convex oval dome evidently accounts for the central ridge seen with transmitted light in potashed preparations¹.

In a series of punctate mapping experiments, forces up to 10 dynes were applied to the larger campaniform sensilla with a tungsten probe of 1 to 2 μm tip diameter, attached to a piezoelectric ceramic bender mounted on an electric resistance force gauge. While observing with a $\times 600$ microscope, the probe was positioned over the sensillum manually with a micromanipulator, but lowered onto it electrically by a d.c. voltage applied to the bender. The bender voltage thus measured the vertical contour of the surface as a precise indicator of probe location (Fig. 1C, points) and also determined the applied force. In addition, a superimposed a.c. voltage at 3 Hz modulated the contact force by 2 to 4 dynes. At responsive points on the cap, the sensory neurone produced modulated bursts of several nerve impulses per cycle, monitored as 100 to 300 μV spikes from pin electrodes through the femur. A PDP-12 computer was used on-line to average the waveforms of bender voltage, contact force, and impulse frequency (as pulse time density), and off-line for subsequent calculations.

As the probe was moved from point to point on the cuticular surface, variations occurred in the modulated force F developed per volt of a.c. signal V applied to the bender $(\partial F/\partial V)_s$, due to variations in the compliance C_s of the cuticular surface. We could thus use $(\partial F/\partial V)_s$ to measure not only the compliance, but also the indentation produced by the stimulus. Surface compliance C_s was obtained as:

$$C_s = B/(\partial F/\partial V)_s - C_p$$

in which B , the free deflexion of the probe tip per volt applied to the bender, and C_p , the compliance of the probe assembly, are independently measured properties of the apparatus. Surface indentation ΔZ was determined from the force and the compliance as:

$$\Delta Z = \int_0^F C_s dF$$

Minimum thresholds for indentation, which occurred at the centre of the dome, were 10 to 20 nm, of the order of 0.03% of the combined length of the dendrite and sensory process of the bipolar neurone⁴. Force sensitivity (impulse frequency modulation per unit force modulation) displayed a broad peak along the major axis of the dome (Fig. 2) and a sharper peak along its minor axis. Indentation sensitivity (force sensitivity divided by compliance) peaked even more sharply,

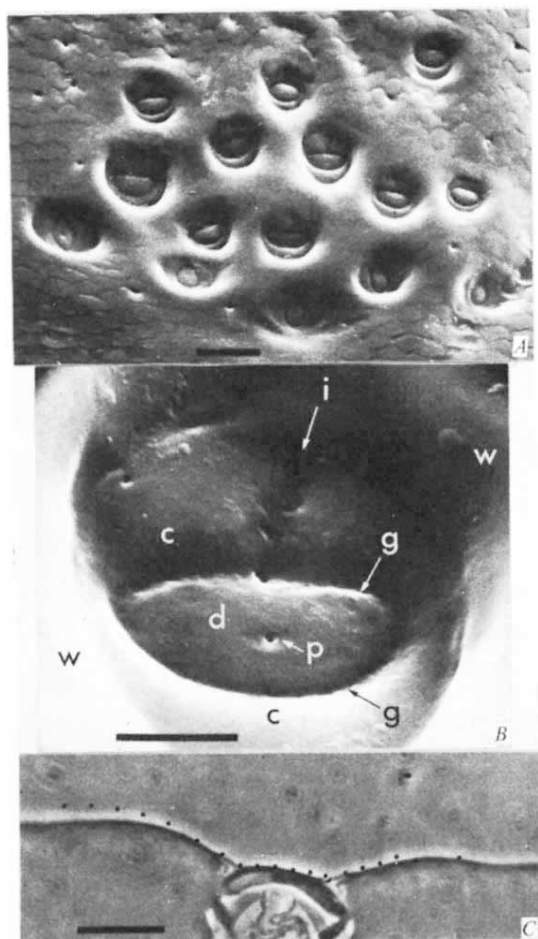


Fig. 1 A and B. Scanning electron micrographs (SEM) of the proprioceptive campaniform sensilla of group six in the leg of the cockroach *Blaberus giganteus*, on the proximal dorsal surface of the tibia. A, Low power SEM, specimen tilted 30° to the electron beam. Fifteen sensilla are seen recessed beneath the tibial surface in crater-like depressions. The top of the picture is proximal. Scale, 20 μm horizontally, 23 μm vertically. B, High resolution SEM of a single sensillum oriented as A. Convex oval dome, d , is separated by narrow groove, g , from the thin surrounding cap cuticle, c , which joins the thick walls of the crater, w . Pit, p , formed around the old sensory process before ecdysis at the last moult. Irregular depression, i , is proximal to the dome regardless of the dome's orientation. Scale, 5 μm horizontally, 5.8 μm vertically. C, Phase micrograph of the section through the major axis of the sensillum of Fig. 2, comparing the anatomic contour of the cuticle with that measured by the stimulating probe. Points are those of Fig. 2, "surface elevation", left. Sensory process and other non-cuticular tissue was not preserved. Scale, 10 μm .

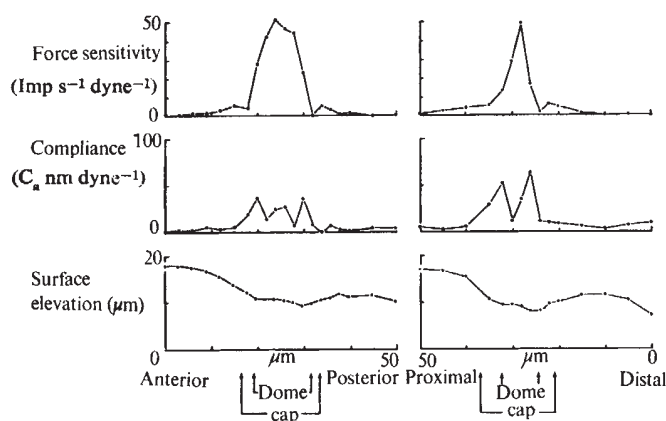


Fig. 2 Profiles of sensitivity, compliance and surface contour from mapping measurements on the major (left) and minor axis (right) crossing the centre of a large transversely orientated sensillum of *B. discoidalis*. High sensitivity to contact force and indentation is restricted to the midline of the dome, while compliance maxima occur on the surrounding groove.

with a maximum of 2 to 5 impulse $s^{-1} nm^{-1}$ at the centre of the dome. The most sensitive region of the cap for stimulating the sensillum, the midline of its long axis, thus corresponds to the position of insertion of the flat, paddle-shaped tip of the sensory process^{4,5}. Greatest compliance, however, occurred at the edges of the dome, forming a ring around it. This suggests that the dome is a relatively hard object suspended on a soft gasket. It probably tilts when probed at its edges, but indents uniformly when probed at its centre. Tilting is thought to excite insect hair sensilla by pinching¹¹ or bending¹² the sensory process, but this is evidently a weak stimulus for proprioceptive campaniform sensilla.

We therefore support Pringle's hypothesis¹ in that the compliance of the cap cuticle surrounding the dome differs indeed from that of the dome itself. Gently indenting the dome, however, is an extremely effective experimental stimulus and should compress the sensory process longitudinally into the dendrite, rather than to stretch¹, pinch¹¹, or bend¹² it. We suggest, therefore, that the cuticular strains produced physiologically during proprioception excite the campaniform sensillum in this way by indenting its dome by a few tens of nanometres.

We thank Dr K. R. Porter for the use of the scanning electron microscope, and Dr J. T. McIlwain for criticizing the manuscript. This work was supported by grants from the US Public Health Service.

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Normal Development of Mechanically Activated, Unlaid Eggs of an Endoparasitic Hymenopteran

In the Ichneumonid wasp *Pimpla turionellae*, as in many other Hymenoptera, males develop from unfertilized eggs with a haploid set of chromosomes. Thus, fertilization of the egg is not necessary for its activation. During our investigations on egg development in *Pimpla*¹⁻⁵ the question arose as to what actually causes the conclusion of meiosis and initiation of cleavage in the oocyte nucleus after oviposition, and also what induces the beginning of the characteristic morphogenetic movements of the ooplasm in initial regions⁵.

In *Pimpla*, the diameter of the ovipositor egg canal (less than 60 μm in its distal part) is much smaller than the width of a fully grown egg (on average 260 μm at the widest point). This implies that on its way through the ovipositor the egg must be squeezed firmly. We assumed that the mechanical deformation of the egg alters the egg architecture and thus induces the beginning of embryonic development, for undeformed eggs explanted from the ovarioles of imago ready to oviposit do not develop. The correctness of this assumption has now been proved by the demonstration that explanted eggs which were pressed through artificial capillaries developed to hatching (Fig. 1).

To obtain eggs the abdomen of female imagoes which showed egg-laying behaviour (for rearing conditions and biology of *Pimpla* see Meng¹) were torn apart in insect Ringer², thereby exposing the ovaries; the eggs were then carefully removed from the ovarioles without pinching. We singled out the eggs which had completed oogenesis, that is eggs which in bright field showed a dark axial column (plasm from the degenerated nutritive chamber³) of 60 μm or less measured at 50% of the egg's length. The eggs were transferred into fine watchmaker's oil (Herm. Koch, Hildesheim, No. 1) where most of the adhering Ringer's solution was removed. With the help of a syringe with microfeed equipment (B. Braun, Melsungen) the eggs were then pressed through a polythene capillary. PVC or glass capillaries could not be used, because the eggs stick in them. The problem of getting the eggs through the fine capillaries without splitting the membranes was solved by using the bone oil mentioned above as lubricant (synthetic oils were unsuitable) and also by pressing the wider anterior egg pole first through the narrow passage, instead of the narrower posterior pole as is the case with the ovipositor. The following procedure gave the best results. The egg was pressed little by little into the capillary, until the egg equator reached the narrowest passage. The pressure was then reduced and the egg allowed to slip back. After this the pressure was increased once more until the equator had passed the narrowest passage. Again the pressure was reduced and this time the whole egg slipped slowly through the capillary. For optimum handling of the eggs air bubbles in the oil between egg and pressure site should be avoided. After the treatment the eggs were replaced in Ringer's solution, where the whole of embryonic development took place.

It is not possible to give the exact dimensions of the capillaries we used, because they were irregular. The capillary which proved to be best, however, is shown in Fig. 1b; its narrowest passage is 95 μm and its length about 4 mm. In 155 eggs pressed through this capillary fifty-four (34.9%) more or less normal (hatching) larvae and nine (5.8%) abnormal embryos developed.

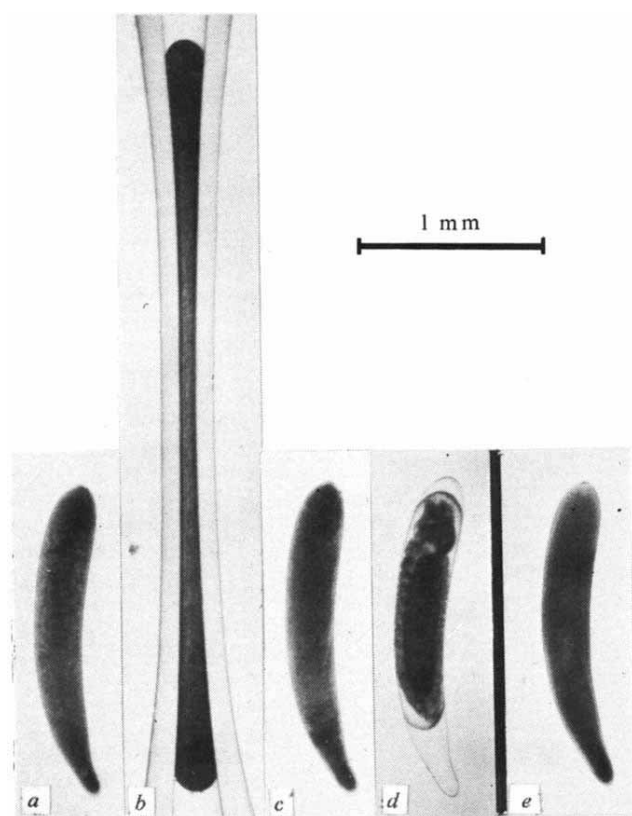


Fig. 1 Unlaidd egg before (a), during (b) and after (c, d) mechanical treatment and egg after oviposition (e). a, Egg architecture after explantation from the ovary, in insect Ringer; b, egg during mechanical treatment in oil; c, egg architecture 4 min after treatment in insect Ringer; d, fully-developed embryo 32 h later; e, egg explanted from host pupa in insect Ringer 4 min after oviposition, for comparison with c. a, c and d show the same egg.

Three of six other capillaries with similar dimensions (smallest passage between 80 and 100 μm ; length between 3 and 6.5 mm) tested with five eggs each also produced embryos or larvae (five of fifteen eggs).

Eggs of unmated females (females which had been isolated immediately after hatching) could be activated with the same success as eggs of mated females. Another reason for assuming that all eggs we handled were unfertilized is that the treated eggs had been explanted from the ovarioles and egg insemination in the imago, however, probably takes place in the oviduct or in the vagina. To make certain that these eggs developed males, we implanted forty-one larvae hatching from artificially activated eggs into *Galleria* pupae (method according to Günther⁴). Five imagos hatched, all males. Four of them were tested for fertility by setting them together with two unmated females each. The females put together with two of the males showed that they had been fertilized by producing female progeny. The other two males were presumably also fertile, but the number of offspring tested (five eggs per female) may have been too small.

In the time between explantation of the eggs and their mechanical treatment they could be stored in the Ringer's solution up to 24 h without drastic changes to the egg architecture. They could be induced to start embryogenesis even then, although in a somewhat lower percentage than if immediately after explantation.

According to von Borstel^{7,8} who assumed that in the parasitic wasp, *Habrobracon*, egg activation is also achieved by the distortion of the egg during egg laying, the position of the egg nucleus is altered during the oviposition. We can give no information as to a possible displacement of the oocyte nucleus resulting from the egg distortion during oviposition or the

mechanical treatment in *Pimpla* as yet, for the nucleus is hard to locate in the egg just before and after the egg is deposited. In any case, the oogenetic architecture of the unlaidd egg obviously has to be altered by oviposition such that the induction and coordination of subsequent developmental processes in the egg, like termination of meiosis, intravittelline cleavage and characteristic ooplasm flows, are guaranteed. Whether this also holds true for eggs of other Ichneumonids and perhaps even of other Hymenopteran families is still open for investigation.

No activation of explanted eggs could be obtained by the following methods: temperature shocks, ultrasonic treatment, osmotic shock such as desiccation of the eggs, transferring them into distilled H_2O or hypertonic salt solutions, mechanical treatment other than pressure through capillaries (such as suction through EM apertures or squeezing between glass plates).

This work was supported by the Deutsche Forschungsgemeinschaft.

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Received May 2, 1973.

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A Carboniferous Insect

AN expedition to search for insects in the Upper Carboniferous freshwater shales of the Hellyer Gorge area of north-western Tasmania in December 1972 was successful. The single specimen obtained is a whole insect (Fig. 1) with wing span of 50 mm, preserving all details of both a fore and a hind wing and some details of the body.

This palaeopterous insect combines characters of the Palaeodictyoptera and Megaseoptera with others unknown in these two orders. The venation is very reduced and closely comparable with that in more generalized megaseopterous wings except that the subcosta ends clearly on the costal margin and slightly beyond the middle of the wing. In contrast to the Megaseoptera, and resembling Palaeodictyoptera, the wings are broad at the base, with the hindwing slightly wider than the forewing.

The body is of simple form, resembling that of Recent stoneflies. Paratergal processes are not present on either the pronotum or segments of the abdomen, as they are in all Palaeodictyoptera and Megaseoptera in which these structures are preserved. The head is slightly longer than wide, and the mouthparts are apparently as long as the head although the structure of this portion of the fossil is confused and possibly includes the basal articles of the antennae. The abdomen appears long due to stretching of the intersegmental membranes during partial break-down of the internal tissues; in life, however, it was probably no longer than the wings.

The palaeoecology of the area of fossilization indicates that the specimen is preserved in the fine sediment deposited at the margins of a small shallow lake adjacent to or in association with glacial moraines. The sediment was almost certainly carried into the lake with the snow-melt. Associated fossils are rare. Plant fronds and pinnules tentatively identified as "*Rhacopteris*" *ovata*, aggregations of a filamentous alga, and an anostracan crustacean have so far been obtained from this series of 1.5 m of shale sediments.



Fig. 1 Fossil insect from the Upper Carboniferous freshwater shales of the Hellyer Gorge in north-western Tasmania.

Abundant animal tracks are preserved in the shale bands of other strata of comparable age in the same area. The shale layers alternate with fine sandstone layers. These tracks of *Tasmanadia twelvetreesi*, described as those of annelid worm¹, and subsequently referred to shrimp-like Crustacea², are most probably those of euthycarcinoid Crustacea.

This Upper Carboniferous insect horizon is an outstanding record as not only is it one of the few known Upper Carboniferous horizons, but it is also the known horizon of this age in the southern hemisphere. (An undescribed specimen from the base of the Permian or very top of the Carboniferous has been obtained from a bore core in Rhodesia.) In addition, it lived in ecological conditions much colder than those known for any other Upper Carboniferous insect horizon, which indicates that, even at this early period in their evolution, the insects had adapted to diverse ecological conditions.

The find almost certainly indicates a land connexion between at least Australia and those areas of the northern hemisphere in which insects of comparable or greater age occur, after the origin of winged insects and their occurrence in the Upper Carboniferous of Australia, because not only were insects present in Australia at this time but also because, from the structure of the wings, this type of insect was not adapted to dispersal over long distances.

A more detailed study of the area is planned, in the hope that a more extensive and richer fauna may be found to add to this significant link in the early history of winged insects.

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Potent Pyrethroid Insecticides from Modified Cyclopropane Acids

THE natural pyrethrins¹⁻³, of which the most important constituent is pyrethrin I (1), are very active against a wide range of insect species, do not leave persistent residues, and have a long record of safe use; despite surprisingly high intravenous toxicity, they have low oral toxicity to mammals⁴. The related synthetic compound, bioresmethrin (2)^{5,6}, has greater insecticidal activity (for example, about fifty times that of pyrethrin I, by topical application⁵, to a normal strain of houseflies), yet is even less toxic to mammals, both orally and intravenously^{4,7}. Pyrethrin I and bioresmethrin are esters of (+)-*trans* [1R,3R]-chrysanthemic acid. We have now found that a simple modification of the acid side chain in bioresmethrin by which the four carbon atoms in the isobutenyl group are redispersed to a but-1-enyl substituent (as in 7) produces still greater insecticidal activity (about 100 times that of pyrethrin I to houseflies) whilst retaining low mammalian toxicity. Other changes of the acid side chain in bioresmethrin increase insecticidal activity further; for example the compound (10) with a *cis*-(Z)-butadienyl group and the dichlorovinyl compound (12) are both 2-3 times as potent as bioresmethrin.

In both insects⁸ and mammals⁹, the *trans* (E)-methyl group (arrow, Fig. 1) of the chrysanthemate side chain in pyrethrin I and related cyclopentenolone esters is a primary centre for metabolic attack. We therefore investigated esters of 5-benzyl-3-furylmethyl alcohol with acids lacking the *trans* (E)-methyl group, hoping thereby to suppress a detoxification route and thus increase insecticidal potency. The ester (4) with a vinyl substituent was less active than the chrysanthemate to houseflies and mustard beetles (Table 1) but the monomethyl compound (5) was more active, particularly to houseflies. Greatest activity in the series to both insects was attained with the but-1-enyl side chain (7), the next two homologues with pent-1-enyl (8) and hex-1-enyl (9) substituents being less toxic.

An even more effective ester (10) (synthesized by the general Wittig route, Fig. 2) had a *cis*-(Z)-butadienyl substituent; this is one of the most potent insecticides known, of any class (LD₅₀ 0.002-0.004 µg per insect).

The structures of outstandingly active synthetic pyrethroids (for example compounds (2), (7) and (10) and the ethanochrysanthemate (3)¹⁰, all stereochemically equivalent derivatives of 5-benzyl-3-furylmethyl [1R] 2,2-dimethylcyclopropane carboxylate) thus support the conclusion⁷ that an appropriately

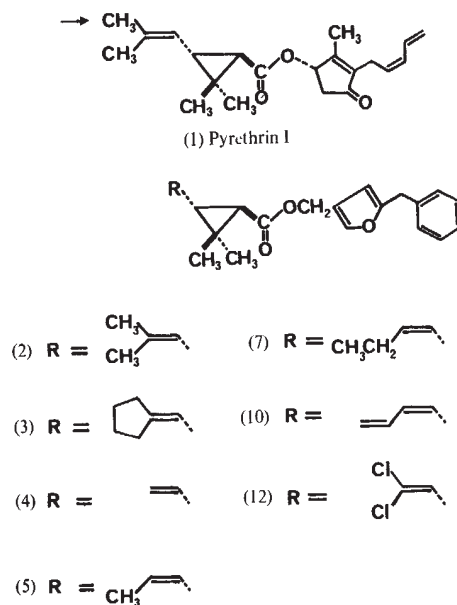


Fig. 1 Pyrethrin I and modifications giving related compounds.

Table 1 Toxicity of Pyrethrin Compounds to Insects and Mammals

	Side-chain (R in second formula)	Relative toxicities* to Houseflies (<i>Musca domestica</i> L.)	Mustard beetles (<i>Phaedon cochleariae</i> Fab.)	Oral toxicity to rats† (<i>Rattus rattus</i>) LD ₅₀ , mg kg ⁻¹
Pyrethrin I	(1)	2	160	260-420 (ref 4)
Bioresmethrin	(2) 2-Methylprop-1-enyl	100‡	100‡	> 8,000 (ref 4)
RU 11,679 ["ethanochrysanthemate"]	(3) Cyclopentylidenemethyl	130	170	100§
	(4) Vinyl	41	65	
	(5) Prop-1-enyl (Z)	120	130	> 800"
	(6) Prop-1-enyl (E)	130	130	
	(7) But-1-enyl (Z)	170	160	800-1,000"
	(8) Pent-1-enyl (Z)	63	71	
	(9) Hex-1-enyl (Z)	27	45	
	(10) Buta-1,3-dienyl (Z)¶	300	300	
	(11) 2-Chlorovinyl	130	130	
	(12) 2,2-Dichlorovinyl	250**	270††	> 400" [(±)-trans isomers]
	(13) 2,2-Dichlorovinyl (50/50 mixture of (±)-cis-trans isomers)	150	190	40"
	(14) 2,2-Dibromovinyl	110	190	

* By topical application of measured doses in 1 µl of acetone on females (houseflies) or day old adults (mustard beetles) then comparison of LD₅₀ values derived from probit analysis.

† Compound administered in glycerol formal (DMSO for compound (1), olive oil for compound (3)).

‡ Taken as standard.

§ Result provided by Roussel-Uclaf.

¶ Results communicated by Mr R. D. Verschoyle, Medical Research Council, Toxicology Unit, Carshalton, Surrey.

|| About 80% Z isomer. The E isomer is less toxic.

** LD₅₀ 0.0028 µg/insect (about 0.14 mg kg⁻¹).

†† LD₅₀ 0.003 µg/insect (about 0.3 mg kg⁻¹).

orientated gem-dimethylcyclopropane unit is essential for high toxicity to insects. To examine further related compounds we synthesized the monochlorovinyl compound (11) (an isostere of (5)) and, finding it more active than the chrysanthemate (2), made the dichlorovinyl ester (12). The 5-benzyl-3-furylmethyl ester (12) of the (+)-trans [1R,3R]-dichlorovinyl acid, obtained by the Wittig route, had exceptional activity, 2-2.5 times as great as that of bioresmethrin. Even the (±)-cis,trans form of the acid, synthesized in excellent yield by a development of an earlier process¹¹, gave an ester with 5-benzyl-3-furylmethyl-alcohol more active than bioresmethrin, which uses the resolved form, (+)-trans-chrysanthemic acid. The ester (14) of the corresponding [1R,3R]-dibromo acid was a little less active, though still very potent.

The compounds described, including the new halogenated derivatives, show that the insecticidal activity of pyrethroids such as bioresmethrin can be increased by small changes in the molecule at sites, such as C-3 on the cyclopropane ring, not directly involved in the poisoning process. The group at C-3 apparently exerts a powerful influence on the level of activity by determining susceptibility to detoxification (cf. bioresmethrin^{12,13}) and physical properties and so rates of transport into and within the insect. Mammalian toxicity is not influenced correspondingly by the change in structure (see Table 1). The trans esters (6), (7) and (12) lack the known specific position of oxidative attack (Fig. 1, arrowed methyl) yet have low oral toxicity to rats, whereas the presence of substituents which sterically hinder esterase action (for example, in the cis component of compound (13) and in the tetramethylcyclopropane

carboxylates⁷) leads to greater mammalian toxicity. Susceptibility to ester cleavage may thus be more important than attack by oxidases in conferring low mammalian toxicity on pyrethroids (compare ref. 13).

The new compounds are protected by the UK Patent Applications Nos. 24809/72, 24811/72 and 30838/72. We thank Dr G. Pattenden for generous gifts of methyl (±)-trans vinyl and prop-1-enyl-2,2-dimethyl-cyclopropane carboxylates¹⁰, used in preliminary work, Dr J. M. Barnes and Mr R. D. Verschoyle for communicating and discussing mammalian toxicity data and the National Research Development Corporation for financial support.

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Received April 11; revised May 4, 1973.

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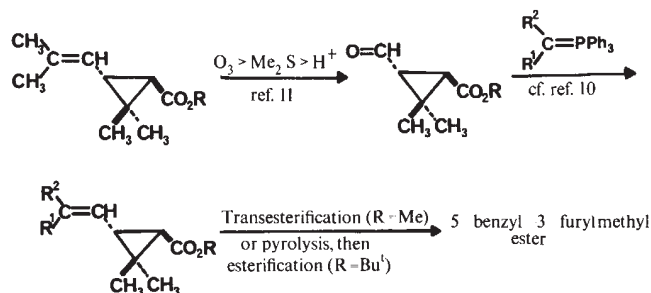


Fig. 2 General Wittig route of synthesis of compound (10).

Experimental Model for Production of Perforating Duodenal Ulcers by Cysteamine in the Rat

It has long been known that exposure to stress, in itself¹ or in combination with various sensitizing agents, particularly glucocorticoids^{2,3}, can produce acute haemorrhagic erosions of the gastric mucosa which have been used as experimental models of "stress ulcers". However, these erosions have little, if any, tendency to perforate and usually disappear after a few days unless the eliciting stressor is fatal before healing can occur.

On the other hand, rats are unusually resistant to the induction of duodenal ulcers and the few techniques developed for their experimental production are, in general, not readily reproducible because of individual variations in sensitivity and the comparative complexity of the procedures used. Until recently the most rapid and reliable method described was that of Robert *et al.*⁴⁻⁶, which depends essentially on the continuous subcutaneous infusion of various secretagogues, such as histamine, carbachol and pentagastrin. Yet, to obtain a high incidence, several of these agents must be administered simultaneously during fasting. Another even more complex means for the induction of duodenal ulcers in the rat is the constant perfusion of the stomach and duodenum with acid after introduction of a permanent catheter through a skin incision in fasted and immobilized rats⁷.

While studying the effect of hormones on resistance to various toxicants⁸, we noted accidentally that acute and almost invariably perforating duodenal ulcers developed in rats given cysteamine-HCl (β -mercaptoethylamine-HCl) either orally or parenterally. Essentially similar results were obtained in the course of these same studies in rats treated with 3,4-toluenediamine⁹ or propionitrile¹⁰, but the ulcerogenic effect of cysteamine is even more rapid and constant, thus providing a particularly reliable model for investigations on the mechanism of duodenal ulcerogenesis and possible means for its prevention.

All our experiments were performed on female Sprague-Dawley rats (Canadian Breeding Farms) with an average initial body weight of 100 g (range 90–110 g). Throughout the period of observation, the animals had free access to Purina Laboratory Chow and tap water.

Cysteamine-HCl (given by the California Foundation for Biochemical Research) was administered at the dose of 100 mg in 1 ml water orally once, or 50 mg in 0.5 ml water subcutaneously once, on the first day to two groups of ten rats each. Preliminary experiments had shown that smaller amounts do not consistently produce duodenal ulcers and similar doses of cysteamine are fatal within minutes or hours when given intravenously or intraperitoneally, and so unsuitable for our purpose.

It is important to emphasize, however, that in the group receiving a single oral dose of 100 mg of cysteamine, mortality within the first 48 h was 70%, and all animals had grossly evident duodenal ulcers. In general, the lesions began with the formation of an oval reddish spot about 3 × 5 mm in size just below the pylorus. Subsequently, this entire region became liquefied and the pyloric contents escaped into the peritoneum (Fig. 1). Yet generalized peritonitis was sometimes avoided through the formation of adhesions between the pylorus and the liver. In these instances, a deep cavity developed in the adjacent hepatic parenchyma as a consequence of erosion by the duodenal digestive juices.

Subcutaneous administration of cysteamine was less consistently effective in producing duodenal ulcers. This is probably due to the fact that 100 mg of the compound, given subcutaneously, caused immediate mortality in most cases, and so we had to limit the dose to 50 mg once subcutaneously on the back. Under these conditions, seven out of ten animals died within 48 h and five of these exhibited perforating duodenal ulcers. Consequently, as a routine model for the production of



Fig. 1 Gross appearance of a perforating duodenal ulcer just distal from the pylorus. The affected region is haemorrhagic and liquefied.

these lesions, only oral administration can be recommended, but it is of theoretical importance to know that the compound does not act merely by direct contact with the duodenum.

It is evident that cysteamine given orally or parenterally is highly active in producing circumscribed and usually perforating duodenal ulcers in rats. This effect is obtained in animals allowed free motion with access to food and water during the entire period of observation. In the course of our systematic studies on the effects of hormones and nonhormonal microsomal drug-metabolizing enzyme inducers on the toxicity of various agents, we have noted the occasional occurrence of duodenal ulcers after poisoning with acetanilide, allylchloride, acetaminophen or 4,4-diaminodiphenylmethane. However, among the compounds tested so far, only propionitrile and 3,4-toluenediamine induced a very high incidence of perforating duodenal ulcers within a few days following oral or parenteral administration^{9,10}.

None of these substances is as reliable as cysteamine in regularly producing perforating duodenal ulcers after a single oral dose, but the fact that so many compounds, widely differing in chemical structure and pharmacological properties, have such a selectively destructive effect on the same segment of the duodenum is noteworthy and will be investigated further. In man, "stress ulcers" are frequently localized in the duodenum but in the rat this is quite exceptional and multiple gastric erosions are the most characteristic gastrointestinal manifestations of exposure to stress. The ability to elicit circumscribed duodenal ulcers in rats is not merely a nonspecific stress effect but, in view of the many agents capable of inducing such lesions, some relation to nonspecific stress may be suspected. Possibly, certain chemical characteristics of the toxicants require "conditioning" by stress factors in order to acquire toxicity for the tissues of this particular region.

As cysteamine was used for experimental purposes by many investigators, it is somewhat unexpected that this particular effect on the duodenum appears to have escaped attention.

In the course of a study on osteolathyrisms, Lalich¹¹ noted that if large amounts of cysteamine were mixed with the diet of rats, they caused loss of weight and "minimal osteolathyrisms". Yet no mention was made of any intestinal lesions.

Special attention was given to cysteamine and related compounds because of their radioprotective effect¹²⁻¹⁸; although in

many of these studies sublethal and even lethal amounts were used, we could find no description of any duodenal lesions.

This work was supported in part by the Medical Research Council of Canada, the Ministère des Affaires Sociales, Quebec, and the Colonial Research Institute, Freeport, Bahamas. S. S. is a fellow of the Medical Research Council of Canada.

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Symbiosis between *Rhizobium* and the Non-legume, *Trema aspera*

ROOTS of *Trema aspera* Decaisne plants growing between rows of tea in the Pangia District of New Guinea showed abundant nodulation, similar to the nodules found on many tropical legumes. Non-flowering nodulated plant specimens were identified by the botanists of the Department of Forests, Lae, New Guinea.

Trema aspera belongs to the family Ulmaceae. Reviews¹⁻⁶ of non-legume plants bearing nodules do not record root nodules on plants in this family. Attempts to isolate and identify the endophytes from these known non-legume nodules have been summarized^{3,6} and indicate that the endophyte has marked affinities with the actinomycetes. Nodulation has been recorded^{7,8} on a further seven plant species of non-legumes in five plant families. "*Rhizobium*-like" organisms have been isolated⁸ from nodules formed on *Artemisia ludoviciana* (Compositae) but the isolates in pure culture were unable to form nodules on plants in their laboratory.

Root nodules (5–10 mm long by 1–1.5 mm diameter) from *T. aspera* were collected on two occasions and culture of the endophyte attempted. Only one type of microorganism grew on yeast-mannitol agar plates inoculated from six nodule squashes on each occasion. The abundant growth indicated that these organisms were present in large numbers. Nodule squashes examined by phase contrast microscopy showed rods resembling the bacteroids of *Rhizobium* in nodules from *Vigna sinensis* and other tropical legumes.

In laboratory culture, the isolated bacteria (strain NGR 231) are rods, mainly straight but sometimes curved, occurring singly, with two refractile areas near the ends of the

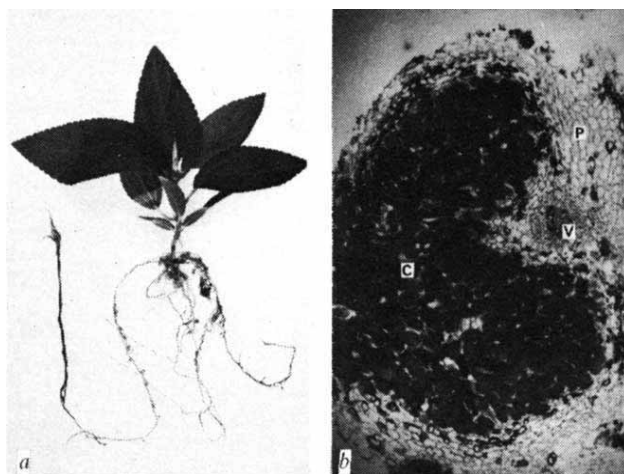


Fig. 1 a, Nodulated *Trema* plant. b, Internal structure of nodule developed on *Trema* following inoculation with strain NGR 231 ($\times 32$) showing cortical parenchyma cells (P), vascular bundles (V) and innermost bacteroid area (C).

cells. Cultures heated at 75° C for 30 min failed to grow, indicating that the *Trema* isolates do not contain spores. The bacterium is motile, Gram negative, and measures 1.7–3.5 $\mu\text{m} \times 0.7$ –0.9 μm . Electron micrographs of young cultures show subpolar flagellation of the bacterium. After 10 d incubation, the colonies produced are circular, 1 mm in diameter, and convex in elevation; the surface is smooth and glistening; it is opaque and white but may develop a brown pigment with age. Cultures utilize dextrin, inulin, maltose, fructose, galactose, glucose, mannose and rhamnose but the preferred carbon sources are arabinose and xylose. Growth in litmus milk results in an alkaline reaction with the formation of a narrow serum zone. These features are typical of slow-growing strains of *Rhizobium* sp.

Strain NGR 231 was tested for its ability to nodulate *T. aspera* (seed supplied by the Department of Agriculture, Stock and Fisheries, New Guinea, from the Tambul District, 80 km from Pangia) and a number of legumes representing the main cross inoculation groups. All species were grown in Leonard jar assemblies, either uninoculated (with and without adequate combined nitrogen) or inoculated with strain NGR 231. In addition, the legumes were inoculated with known effective strains of *Rhizobium*. All treatments were in triplicate, with two plants per assembly.

Trema formed nodules in response to inoculation with strain NGR 231. The nodules were effective in nitrogen fixation, as shown by the strong growth of nodulated plants (Fig. 1a) in a rooting medium devoid of combined nitrogen. Acetylene reduction assays for nitrogenase activity^{10,11} gave positive results with nodulated whole plants and detached nodules, but not with roots without nodules (Table 1) indicating that N_2 fixation was restricted to the nodule. The low value for detached nodules parallels the diminished activity of detached legume nodules^{11,12}. The mature nodules were often 7 mm in length, loosely attached to the root and were elongate with branching (see ref. 13).

All uninoculated legume plants were free of nodules while effective nodulation with strain NGR 231 was obtained on plants of four species (*Vigna sinensis*, *V. sinensis* ssp. *sesquipedalis*, *Macroptilium atropurpureum* (formerly *Phaseolus*

Table 1 Acetylene Reduction Assay of *Trema aspera* Nodules and Root Tissue Following Inoculation with Strain NGR 231

Material assayed	$\mu\text{mol C}_2\text{H}_4 \text{ g}^{-1} \text{ nodule}$ fresh wt h^{-1}
Nodulated plant 1	11.5
Nodulated plant 2	11.7
Detached nodules (6)	4.6
Nodule-free roots (2)	0

atropurpureus), and *M. lathyroides* (formerly *P. lathyroides*)). The nodules were pink, large, and typical of the nodules produced by effective "cowpea-type" rhizobia¹³. The fifteen species which failed to nodule included *Lablab purpureum* (formerly *Dolichos lablab*), *Crotalaria anagyroides*, *Glycine max*, *Acacia farnesiana*, *Phaseolus vulgaris*, *Trifolium pratense*, and *Medicago sativa*.

Haematoxylin stained sections of NGR 231 nodules formed on the four legumes were structurally similar to the nodules produced by authentic *Rhizobium* strains on these species. The structure of the nodules formed on *Trema* resembled the overall histological pattern of legume nodules, and thus was quite unlike that of non-legume nodules such as those of Alder. Cortical parenchyma cells, a peripheral vascular system and an innermost bacteroid area consisting of plant cells packed with bacteria, are shown in Fig. 1b.

Cultures isolated, and bacteroids, from nodules on glass-house grown plants of *Trema* and legumes were specifically agglutinated by the antiserum for NGR 231. The specific reaction only occurred after the antigen was heated at 100° C for 1 min¹⁴. The serological specificity of NGR 231 antiserum was demonstrated by its failure to agglutinate any of forty-three *Rhizobium* strains originating from nodulated legumes grown in New Guinea and representing a number of cross inoculation groups.

The probable phylogeny and relationships of the Orders of Angiosperms, as given by Hutchinson¹⁵, place the Urticales (of which the family Ulmaceae is a member) in the same evolutionary line as other nodule bearing Orders. My results show that *Trema aspera* may now be added to the list of nitrogen fixing nodulated plants, that the endophyte is a bacterium (which is of interest in that most endophytes of non-legume nodules have so far defied isolation and precise identification) and that the bacteria satisfied the criteria necessary to classify them as *Rhizobium* species. The *Trema* root nodule bacterium is the first of the rhizobia known to effectively nodulate a non-legume.

The initial observations, the isolation of the endophyte and preliminary inoculation trials were done whilst I was an officer of the Department of Agriculture, Stock and Fisheries in New Guinea.

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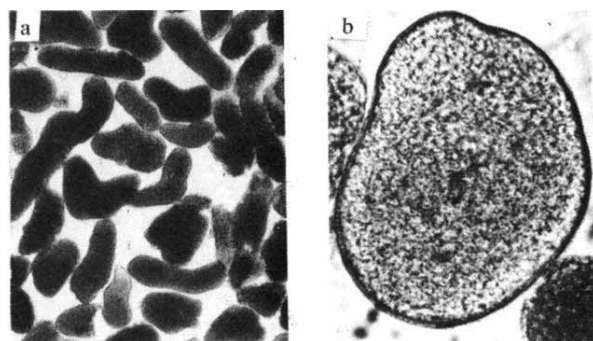


Fig. 1 a, Freshly isolated soybean root nodule protoplasts. Light micrograph $\times 20$. b, Single root nodule protoplast. Bacteroids in the cytoplasm give the protoplast a granular appearance. Light micrograph $\times 80$.

nitrogen fixation². It has, however, been impossible to isolate single cells from infected nodules. Such infected nodule cells would be particularly useful for biochemical studies on nitrogen fixation of infected cells *in vitro*. Recent studies on the somatic hybridization of plants by the fusion of isolated protoplasts suggest that hybridization between legumes and non-legumes may afford a means of transferring the genetic capability of symbiotic nitrogen fixation from legumes to non-legumes³. Protoplasts can be readily isolated from leaves and many other plant tissues using cell wall degrading enzymes, and these naked single cells can often be readily cultured *in vitro*⁴. We have therefore investigated the possibility of isolating protoplasts from legume root nodules.

The seeds and seedlings of soybean (*Glycine max* var Grant) were inoculated with *Rhizobium leguminosarum* (Rothamsted Culture Collection No. 3407). Bacteria for inoculation were cultured as previously described⁵. Young root nodules 4–8 mm in diameter were selected from 16–24 week old plants, surface sterilized in 10% v/v 'Domestos' (Lever Bros. Ltd) with 0.5% v/v 'Teepol' (BDH Chemicals Ltd) for 20 min, and washed with eight changes of sterile water. Nodules (1 g fresh weight) were cut into slices (~ 0.3 mm thick) and incubated in 10 ml of a 'Millipore'-filtered enzyme mixture of 1.5% 'Cellulase' R10, 0.5% 'Macerozyme' R10 (Kinki Yakult Manufacturing Co. Ltd, Nishinomiya, Japan) with 1.0% 'Rhozyme' HP 150 (Rhom and Hass Co. Ltd, Philadelphia) in 0.65 M mannitol (pH 5.8) for 5 h at 20° C in the dark with gentle agitation on a horizontal shaker (70 r.p.m.). The material was then teased apart and incubated without shaking for 1 h. The protoplast-enzyme mixture was strained through a fine wire gauze to remove debris. Protoplasts were allowed to settle, the supernatant removed, and the protoplasts washed with six changes of 0.65 M mannitol. Protoplasts were fixed for electron microscopy in 3% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.0) containing 0.65 M mannitol for 16 h at 22° C. Further stages of preparation were as already described⁶.

Almost complete digestion of the pink-infected nodule tissue occurs using the enzyme mixture described, and $0.6\text{--}1.0 \times 10^6$ protoplasts per g fresh weight of nodule material are freed. Protoplasts are also liberated using only 'Cellulase' with 'Macerozyme', but tissue maceration and protoplast release are stimulated by the addition of 'Rhozyme'. Most protoplasts were elongated (Fig. 1a) and usually retained the shape of the infected cells in the nodule tissue. Failure to become spherical probably results from the large number of bacteroid-containing vesicles in the cytoplasm (Figs 1b, 2a, b) and the absence of a large central vacuole. Thin sections examined electron microscopically showed no cell wall material present at the plasmalemma surface (Fig. 2b). 50–60% of the soybean nodule protoplasts appeared viable on isolation as judged from their ultrastructural appearance and fluorescence when viewed optically using fluorescein diacetate⁶. When the plasmolyticum was reduced to 0.5 M mannitol the protoplasts burst releasing free bacteroids and also vesicles containing bacteroids (Fig. 2c).

Isolation of Legume Root Nodule Protoplasts

INTEREST has developed in the use of cultured cells for establishing symbiosis between *Rhizobium* and plant cells *in vitro*⁴, and in the genes controlling nodulation and the regulation of

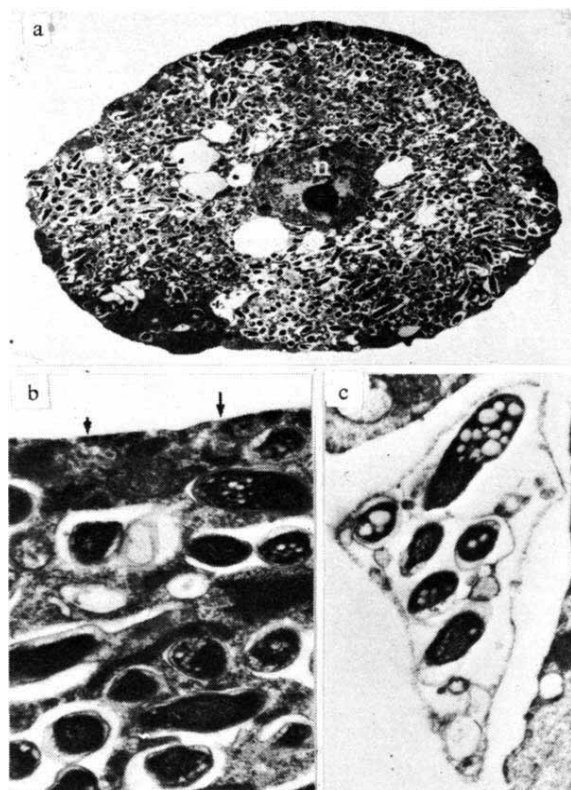


Fig. 2 *a*, Low magnification electron micrograph of a root nodule protoplast. Many bacteroid-containing vesicles are present in the cytoplasm. Nucleus with nucleolus (n). $\times 1,500$. *b*, Electron micrograph of the peripheral region of an isolated root nodule protoplast. The plasma membrane (arrowed) is free of cell wall material. Bacteroids are present in vesicles in the cytoplasm. $\times 15,000$. *c*, Electron micrograph of a membrane-bounded vesicle, enclosing bacteroids, released from a burst root nodule protoplast. $\times 11,000$.

Protoplasts have also been isolated using this procedure from young root nodules of lupin (*Lupinus polyphyllus hybrida*).

As already indicated, these isolated root nodule protoplasts should be particularly useful for biochemical and somatic hybridization studies in connexion with nitrogen fixation. Moreover, vesicles containing bacteroids, which have been suggested as "nitrogen fixing units", and which have been seen to be released from burst nodule protoplasts, may also be of use in these studies.

This work was supported by a Royal Society Pickering research fellowship to M. R. D., and by the Lord Rank Research Centre, High Wycombe. We thank Dr R. Summerfield for soybean seeds, and Dr P. K. Evans for advice on fluorescein staining.

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Effect of Dibutyryl Cyclic AMP and Other Nucleotides on Nitrogen Fixation by Legume Root Nodules

Cyclic AMP mediates reactions in tissues of animals, plants and microorganisms (for reviews see refs 1 and 2). Here we report that dibutyryl cyclic AMP inhibits the fixation of atmospheric nitrogen by nodules of three legumes, *Trifolium repens*, L. (white clover), *Medicago sativa*, L. (alfalfa or lucerne) and *Lotus pedunculatus*, Cav. (lotus). On the other hand, we have found that free cyclic AMP and AMP enhance nitrogen fixation by these nodules.

The rate of acetylene reduction was used as a measure of nitrogen fixation because of its high sensitivity and because data indicate that it correlates closely with nitrogen fixation^{3,4}. Plants were grown from surface-sterilized seed placed singly under sterile conditions in test tubes containing 10 ml of agar medium⁵. The tubes were kept in a glasshouse where light was equally distributed. Ten days later the seedlings were inoculated with the appropriate strain of *Rhizobium* sp. suspended in 1 ml of water. When nucleotides were used the rhizobia were suspended in 1 ml of a solution containing 10 μ mol of nucleotide in most experiments. Seven weeks after inoculation the rate of acetylene reduction was determined^{4,6}. Each result was assessed from the average of those for ten studies.

Table 1 Effect of Dibutyryl Cyclic AMP and AMP on the Rate of Acetylene Reduction by Legume Nodules

Legume	Rate of acetylene reduction nmol per plant h ⁻¹		
	Control	+ Dibutyryl cyclic AMP	+AMP
Clover	15.6	1.1	31.2
Lotus	11.3	2.0	33.5
Alfalfa (lucerne)	21.6	0	29.7

The results in Table 1 show the effect of dibutyryl cyclic AMP and AMP on the rate of acetylene reduction by nodules formed by each of the three legumes. Although the plants treated with dibutyryl cyclic AMP formed nodules, the rate of acetylene reduction by these nodules ranged from an unmeasurable level in alfalfa to 19% of that of the control in lotus. The rate of acetylene reduction by nodules on plants treated with AMP was 30–200% greater than that observed in control plants.

The decrease of the rate of nitrogen fixation in the presence of dibutyryl cyclic AMP could be due to the action of the nucleotide on (1) the host plant, (2) the growth of rhizobia or (3) the process of invasion by rhizobia of the host cells and the subsequent transformation of the non-nitrogen fixing rhizobia to the nitrogen fixing bacteroids in the host cells. Experiments were therefore carried out with white clover to study the effect of dibutyryl cyclic AMP on the growth of plants supplied with potassium nitrate and therefore not dependent for their nitrogen supply on symbiotic fixation of atmospheric nitrogen. These experiments were also planned to compare the effect of dibutyryl cyclic AMP with that of free cyclic AMP, AMP and guanosine monophosphate (GMP) on growth and nitrogen fixation.

When dibutyryl cyclic AMP was present the rate of growth of plants (expressed as fresh weight) which depended for their nitrogen on symbiotic nitrogen fixation decreased in proportion to the concentration of the nucleotide. On the other hand, the rates of growth of plants grown in media containing potassium nitrate were not affected by dibutyryl cyclic AMP (Fig. 1 and Table 2). These results indicate that the inhibition of growth by dibutyryl cyclic AMP was not due to an effect on the host plant when the plant was not dependent on fixation of atmospheric nitrogen.

Dibutyryl cyclic AMP decreased the rate of nitrogen fixation and plant growth whereas free cyclic AMP, AMP or potassium nitrate (nitrogen equivalent to that in 10 μ mol AMP used)

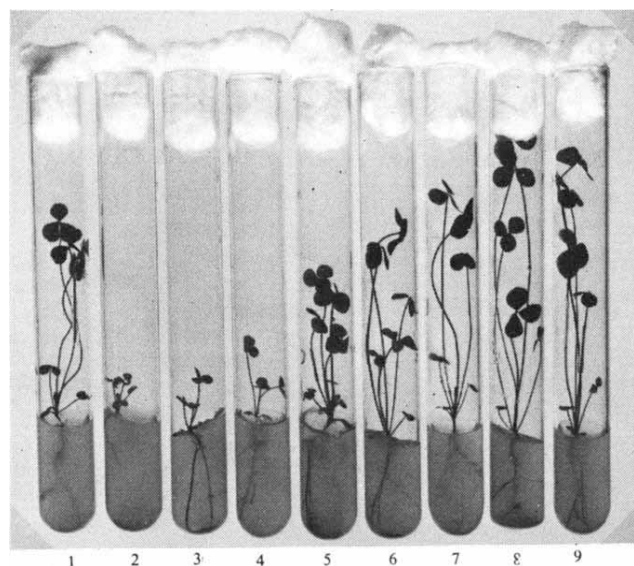


Fig. 1 1, Control; 2, +dibutylryl cyclic AMP (10 μmol per tube); 3, +dibutylryl cyclic AMP (5 μmol per tube); 4, +dibutylryl cyclic AMP (2.5 μmol per tube); 5, +potassium nitrate+dibutylryl cyclic AMP (10 μmol per tube); 6, +potassium nitrate alone; 7, +cyclic AMP; 8, +AMP; 9, +GMP.

increased the rate of nitrogen fixation and plant growth. The enhancement of growth and nitrogen fixation by free cyclic AMP, an opposite effect to that observed with the dibutylryl derivative, may be due to the rapid breakdown of free cyclic AMP by the phosphodiesterase. The product of this degradation, AMP, increased nitrogen fixation and plant growth (Tables 1 and 2). It has been reported⁷ that bromoderivatives of cyclic AMP are far more resistant than free cyclic AMP to degradation by plant phosphodiesterase.

Table 2 Effect of Dibutylryl Cyclic AMP, Cyclic AMP, AMP and GMP on Rates of Growth and Acetylene Reduction by White Clover Nodules

Treatment	Rate of acetylene reduction nmol per plant h ⁻¹	Average fresh weight of plant (mg)
Control	16.6	90
+dibutylryl cyclic AMP (10 μmol per tube)	1.1	10
+dibutylryl cyclic AMP (5 μmol per tube)	1.9	20
+dibutylryl cyclic AMP (2.5 μmol per tube)	4.7	30
+cyclic AMP (10 μmol per tube)	20.8	130
+AMP (10 μmol per tube)	31.2	190
+GMP (10 μmol per tube)	16.2	180
+N (equivalent to amount in 10 μmol AMP) added in the form of potassium nitrate	26.6	210
+N ₂ (equivalent to amount in 10 μmol AMP)+dibutylryl cyclic AMP (10 μmol per tube)	0	190

The increase in the rate of nitrogen fixation by AMP may be due to either a specific effect of this nucleotide on nitrogen fixation or the supply of minute amounts of nitrogen which had been shown to increase the rate of nitrogen fixation in several legumes (unpublished data of A. H.). This was confirmed in the work described here by the increase in nitrogen fixation in the presence of potassium nitrate containing an equivalent amount of nitrogen to that in AMP used (Table 2). Guanosine monophosphate (GMP), unlike AMP or free cyclic AMP, had no effect on nitrogen fixation but it increased the rate of plant growth (Fig. 1 and Table 2). This is difficult to explain by the data presented here.

Preliminary experiments to study the effect of dibutylryl cyclic AMP on the growth of the rhizobial strains used in this work showed that this nucleotide did not reduce the rate of growth of these bacteria when cultured on yeast mannitol agar media⁸.

The results of this work thus showed that dibutylryl cyclic AMP reduced the rate of nitrogen fixation by legume root nodules and that this effect was not exerted by affecting the growth of the host plants or rhizobia when either were grown independently. The effect of dibutylryl cyclic AMP may be at the level of transformation of rhizobial cells into nitrogen fixing bacteroids after invasion by the bacteria of the host plant root cells.

The amounts of cyclic AMP and dibutylryl cyclic AMP used in this work are 10–100 times the normal physiological range, but the amounts of these nucleotides transported across membranes of rhizobial and plant cells remain to be determined.

We thank Dr D. W. Dye for valuable discussions.

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Received May 29; revised July 3, 1973.

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Epidemic Spread of a Rust imported for Biological Control

IN 1971 the autoecious rust fungus *Puccinia chondrillina* Bubak and Syd. was imported into Australia from Europe, an important step in the programme for the biological control of skeleton weed *Chondrilla juncea* L., an extremely serious weed of cultivation in south-east Australia¹. This followed three years of intensive testing and discussion^{2,3}, and represented a highly significant event in Australian quarantine policy. We believe that this is the first example in the recent history of quarantine controls, of the deliberate introduction and successful release in a new country of a plant pathogen for weed control.

The strain introduced was selected on the basis of its virulence against the form of *C. juncea* (an apomict) widespread in Australia and was isolated from a form of this species in Italy seemingly identical with the common Australian form. Following satisfactory demonstration of specificity, this strain was established in quarantine in Canberra and was released in mid 1971 after screening.

Figure 1 shows the distribution of *C. juncea* in Australia and the location of release sites. Where distribution is practically continuous, densities in disturbed land often exceed 100 plants m⁻². The sites are representative of the different environments colonized by the weed and, at several,

work had already been carried out on the population dynamics of *C. juncea*⁴. The climate ranges from dry, predominantly winter rainfall regions in the west (some with irrigation) through a region of increasing rainfall to predominantly summer rainfall areas in the north. Field infections were established at all sites at different times from June to December 1971, but the first successful field infection in Australia was made at Wagga.

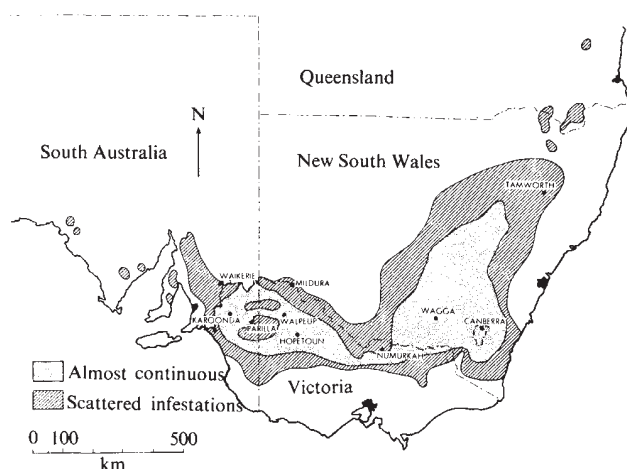


Fig. 1 The present distribution of skeleton weed *Chondrilla juncea* L. in Australia.

On June 10, 1971, nine plants infected with *P. chondrillina* (100–200 uredosori each) were transplanted into the centre of a 0.1 ha plot containing a dense growth of *C. juncea* (143 ± 19 rosettes m^{-2}). Subsequent events are related to an approximate time scale in generations based on the known relationship between minimum generation time and temperature⁵, combined with air temperature records for Wagga.

At slightly over one generation time a few uredosori (about 50 total) were observed on plants close to the transplants. Most were within 20 cm but one was found 1 m distant. With additional infections by spores from the transplants the intensity of this initial secondary infection had increased markedly when inspected just before the completion of the second generation. When this plot was next examined, in early November, after the fifth completed generation, it was apparent that conditions had been favourable for the pathogen. The majority of plants in the plot were infected, some severely (500–2,000 uredosori per plant), and uredosori were found up to 27 m from the centre of the plot. This focus, though still well defined, was obviously large enough to produce infections some distance away.

Two generations later, stands of *C. juncea* were searched 8, 16 and 24 km distant in different directions. Foci could be found in all stands examined at 8 km and at two foci the infection was estimated to be in its third generation. At 16 and 24 km foci were found in 50% of the areas searched, each focus being judged to be two generations old. It was therefore apparent that infections had been started at least 8 km away, four generations after the original transplants were introduced and, similarly, at least 24 km away after five generations. This initial stage of development seems to have been on a scale only slightly less than that recorded for wheat stem rust epidemics^{6,7}.

The spread continued to be rapid thereafter, and it was possible to trace the course of the epidemic with the aid of individual sightings, questionnaires and field surveys.

Foci at minimum distances of 80 km, 160 km and 320 km were probably initiated at the eighth, tenth and twelfth generations approximately, after the initial introduction at



Fig. 2 Distribution of positive and negative records of *Puccinia chondrillina* as at March 31, 1972. ●, Positive identification by March 31; ○, confirmed negative record at March 31; x, later positive identification (only significant records shown).

Wagga. One hundred kilometres to the east and 320 km to the north would probably have been the limits of independent spread, the distribution thereafter overlapping those spreading from Canberra and Tamworth where initial infections were established later. By late March 1972 there was only one undoubted negative record in all stands of *C. juncea* examined in New South Wales, including one of the northerly isolated areas.

In the southern and western region of *C. juncea* distribution, the drier summer produced a different situation. Following initial establishment of foci at considerable distances from some sites, spread was curtailed. Surveys in March 1972 showed an irregular pattern of presence and absence which contrasted with the general distribution in New South Wales (Fig. 2). Following autumn rains in March and April, however, further surveys indicated a general distribution here also.

It was not possible to examine the small isolated areas of *C. juncea* well outside of and to the north, south and west of the main range until varying intervals later, but all were known to be colonized by *P. chondrillina* by December 1972 at latest.

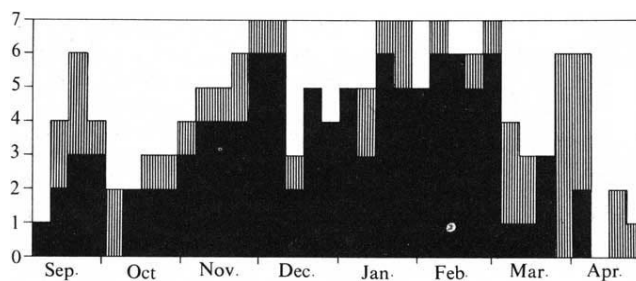


Fig. 3 The number of nights per week when infection by *P. chondrillina* was successful in the field. 1971–1972. ■, Massive infection; ▨, at least some infection.

In New South Wales, above-average rainfall made the summer suitable for fungal infection. A daily survey of the suitability of meteorological conditions for infection has been carried out at Canberra using spore-dusted plants exposed for 24 h periods in the field, and the results of this bioassay for the period of major spread are shown in Fig. 3.

Apart from lower overnight temperatures in spring,

Canberra experienced rainfall and temperature conditions very similar to those of Wagga 150 km away. Winds were predominantly easterly but there were also significant northerly and south-westerly currents.

The plant disease epidemic seen on this occasion has the unique double distinction of being observed on a grand scale and of being both deliberate and beneficial. The density of the host, the virulence of the pathogen and the weather conditions together ensured its success. It is of historic interest that the major spread was from Wagga which was the centre of dispersal of the host *C. juncea*⁸ 60 yr earlier.

The assistance of Mr G. R. Wearne is appreciated. This programme is supported by funds from the Wheat Industry Research Council of Australia.

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Received April 16; revised June 4, 1973.

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Filaments but no Membranous Transcellular Strands in Sieve Pores in Freeze-etched, Translocating Phloem

PLANT physiologists agree that organic substances are transported long distances in plants through sieve-tube elements and through pores in the sieve plates between them. But they disagree about how this transport is driven; sieve tubes might be passive pipes between source and sink or might contain pumps all the way along. This controversy drags on because microscopists suspect that the contents of sieve elements may be altered or displaced as they are prepared for microscopy; thus their *in vivo* configuration remains uncertain. No electron micrograph has yet been published of a sieve element confirmed as translocating when it was fixed.

The outstanding question has been "What is in pores in sieve plates between translocating sieve elements?"¹⁻⁴. In the electron microscope sieve pores often appear to contain closely packed filaments ("p-protein"). Weatherley⁵ has calculated that if these are normally closer than 1000 Å then their resistance to flow will be so high that Münch's simple pressure-flow hypothesis is not adequate to account entirely for translocation; pumps would be needed at places along sieve tubes. Furthermore, he calculated⁵ that the pressure-flow hypothesis might be feasible if filaments in the lumen of sieve elements are bundled together but that if they are uniformly dispersed it would require a

pressure gradient larger than that available in the plant. Thus we need also to know whether filaments in the lumen of translocating sieve elements are dispersed or arranged in separate bundles.

Thaine and his co-workers^{6,7} concluded from light microscopy that "transcellular strands of cytoplasm" lie through sieve pores and provide pathways for translocation. In an earlier paper⁸ Thaine stated that, theoretically, such transcellular strands should be enclosed by a membrane which he believed "to have structural and physiological properties comparable with the tonoplast". More recently he has speculated on the probability of transcellular strands with a contractile structural boundary formed from one or more rings of protein filaments which enclose endoplasmic tubules and perhaps spherosomes and plastids⁹. He speculates that endoplasmic tubules, each about 1 µm wide, might occupy about 70% of the area of cross section of each strand and might be connected to the endoplasmic reticulum along the side walls and at the ends of sieve tubes. He suggests that such hypothetical transcellular strands could drive translocation by peristalsis.

Arguments for true membranes around transcellular strands in sieve elements have come from light microscopy and from analogy with transvacuolar strands in other kinds of cells⁸. But the light microscope does not resolve membranes in cells and cannot, therefore, be used to identify them with certainty in sieve elements. Membranes have never been demonstrated around or within elaborate transcellular strands in sieve elements as proposed by Thaine⁹. At most, some narrow cisternae of endoplasmic reticulum have been shown in sieve pores in some plants¹⁰⁻¹². Thaine⁹ suggests that his membranous transcellular strands fail to appear in the electron microscope because they are degraded by the methods commonly used to fix, dehydrate, or embed them.

Jarvis and Thaine⁷ thought that "progress might be made if sieve tubes were fixed rapidly by freezing rather than by chemical means". They froze intact vascular bundles rapidly, sectioned them in a cryostat, thawed the sections rapidly, and examined them in 1% glutaraldehyde under the light microscope. They show clear photographs of stainable strands, 5-9 µm wide in a sieve element, and interpret them to have a "discrete boundary enclosing groups of parallel substructures".

Previously, Johnson³ had found filaments but no sign of membranous strands in sieve pores in vascular bundles which were frozen rapidly while intact between leaf and plant and then freeze-etched for electron microscopy. The vascular bundles freeze-etched by Johnson³ or those sectioned and fixed by Jarvis and Thaine⁷ might not, however, have been translocating before they were frozen.

Here, I describe electron microscopy of freeze-etched sieve elements and sieve pores in vascular bundles which were translocating ¹⁴C. These bundles were frozen intact to below -50° C in less than 0.03 s.

Whole plants of *Nymphoides peltata* (S. G. Gmel.) O. Kuntze, a water plant with floating leaves, were arranged with their long petioles extended across a flat platform. The leaves were illuminated in separately transparent chambers. Epidermis and aerenchyma were dissected from each petiole from about 6 cm to 11 cm below the leaf leaving the central vascular bundle only. More than 1 h after dissection ¹⁴CO₂ was applied to the leaves for 10 min. The petioles were left for a further hour to translocate, and the exposed vascular bundle of each petiole was then gently but rapidly immersed in liquid Freon 22 at about -145° C. The frozen segment was broken from the plant and transferred to liquid nitrogen. Samples of petiole below it were measured for radioactivity with a Packard Tricarb liquid scintillation spectrometer. Only those pieces of frozen vascular bundle which proved to have translocated normally were used. They were stuck to gold

disks whilst still below -70°C and freeze-etched as described by Johnson³. No fixatives or cryoprotective substances were used.

The time taken for ^{14}C to reach the youngest leaves showed that transport velocity can exceed 40 cm h^{-1} .

Figure 1 shows part of a freeze-etched sieve plate between two mature sieve elements, and part of an adjacent parenchyma cell. Banded filaments (arrows) appear in the fractured sieve pore. Some filaments appear on either side of the sieve plate (arrowheads) but because they tend to be obscured by ice crystals and frozen-out solutes (Z) it is not possible to say how uniformly they are distributed in the lumen. The distance between the apparent centres of filaments in the pores varies between about 100 Å and 500 Å. The diameter of filaments in pores on replicas appears to be between 100 Å and 250 Å; they cannot be distinguished from their shadows. But in sectioned and embedded material their diameter was measured more precisely as 163 Å (s.e.m. 3.2 Å, $n=79$) (Johnson³). Thus the space between the filaments in the pores is, at most, 500 Å. If this spacing is typical of translocating sieve pores then the pressure-flow hypothesis is inadequate^{4,5}. So far I have seen twenty-nine pores in eleven sieve plates, and ten of these pores contained filaments spaced much as those in Fig. 1, eight pores contained some filaments whose spacing could not be determined, and eleven pores were not fractured or shadowed clearly enough inside to show whether they contained any filaments.

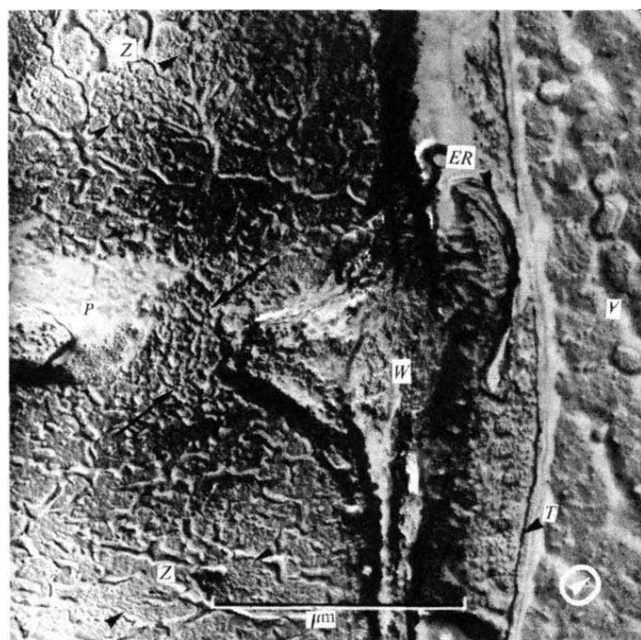


Fig. 1 Part of a sieve plate with a fractured sieve pore containing banded filaments (arrows), filaments nearby in lumen (arrowheads), plasmalemma (P), frozen-out solutes (Z), and part of an adjacent parenchyma cell with cell wall (W), vacuole (V), tonoplast (T), and endoplasmic reticulum (ER). The encircled arrow shows in which direction platinum carbon was applied.

There was no sign of any membranous transcellular strands as proposed by Thaine. Neither did any membranes which could be interpreted as boundaries or inner parts of such strands appear in the lumen of any sieve element. There was no sign of a tonoplast-like membrane in sieve elements^{8,10,13} though tonoplast and endoplasmic reticulum appeared in nearby parenchyma cells (Fig. 1, T and ER). But fractured plasmalemma with particles did appear in surface view on sieve plates and lining sieve pores (Fig. 1,

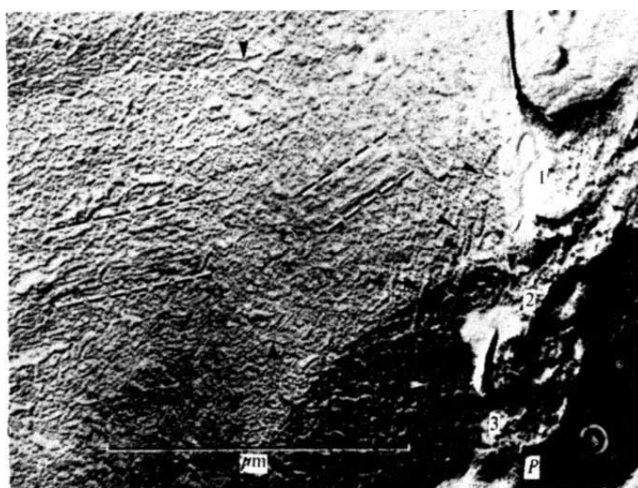


Fig. 2 Part of a sieve plate showing parts of three pores (1, 2, 3) with banded filaments near pores (for example, at arrowheads) and bundles or strands of filaments in the lumen (between broken lines). P, Plasmalemma.

2, P). Also, membranes of plastids appeared clearly in sieve elements (the plastids, not shown here, are about twice the diameter of the sieve pores and too large to pass through them). If there had been membranes in or around any transcellular strands lying through freeze-etched sieve pores in the study described here, they would have been obvious; transvacuolar membranes are obvious in freeze-etched phloem parenchyma cells even when the cytoplasm and vacuoles are distorted by large ice crystals (ref. 3, Fig. 15). Membranes in freeze-etched cells generally show up clearly whether fractured across or along their surfaces¹⁴.

Short bundles of filaments appeared near some sieve plates (Fig. 2, between broken lines), sometimes with separate filaments nearby (for example, at arrowheads). Such short pieces of bundle would appear if the long strands shown under the light microscope by Thaine and his co-workers^{6,7} are, in fact, bundles of parallel filaments. In the electron microscope such bundles would only show their full length if they happened to run exactly along the plane of a fractured surface, or within the thickness of a thin section. The bundles seen (Fig. 2) had a boundary only in the sense that a hank of hair has a boundary. Their appearance does not support suggestions^{4,8,9,15} that filaments in sieve elements might be organized to form semipermeable, or peristaltic, membrane-like tubes.

There now seems little doubt that fine bundles (strands or fibrils) of parallel filaments do occur in sieve elements^{13,16-18} and that these are the transcellular strands seen under the light microscope. My study shows that they can occur in sieve elements that are undamaged by fixatives or release of turgor pressure. The function of these bundles remains obscure, though they have been likened^{4,17-19} to fibrils thought to promote movement in other kinds of cells.

As membranous transcellular strands fail to appear in *N. peltata* in mature sieve elements in vascular bundles frozen rapidly while translocating, it seems unlikely that translocation generally depends on such structures. The notion that transcellular strands in sieve elements are bounded by membranes or contain elaborate systems of membranous tubules essential to translocation should be discarded. It has been premature to promulgate speculative mechanisms based on hypothetical membranous transcellular strands for which there is no evidence.

The close spacing of filaments in sieve pores shown here confirms the close spacing seen in carefully prepared sections^{1,3,12}. It casts doubt on the adequacy of the Münch

pressure-flow hypothesis and suggests that there must be pumps at places along sieve tubes.

I thank Mr D. A. John, Mr I. McRae, and Mr C. Verdan for technical assistance and Dr B. T. Watson for help with ^{14}C . This work was supported by a grant from the Science Research Council.

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Received March 12, 1973.

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Rapid Nitrosation of Phenols and its Implications for Health Hazards from Dietary Nitrites

THE use of sodium nitrite as a food preservative is under close scrutiny because its presence may result in the formation of secondary N-nitrosamines, which are powerful carcinogens in laboratory animals^{1,2}. They may form either in the food itself during processing³ or in the digestive tract from suitable amino compounds⁴⁻⁶. Only minute amounts of secondary N-nitrosamines, however, have been detected in preserved food so far⁷.

But another reason for careful consideration of dietary nitrite levels arises from an interaction with phenolic compounds, again either in food processing or in the digestive tract. The carcinogenicity of aromatic nitroso compounds has not been widely examined, but *p*-nitrosophenol is listed by the Chester Beatty Research Institute as potentially dangerous⁸ (possibly because of its metabolic reduction to an hydroxylamine), and preliminary evidence suggests that 1-hydroxy,2-nitronaphthalene causes hepatomas in rats⁹. Unfortunately, there is less information about the carcinogenicity of nitrosophenolic compounds than for nitrosamines, but there is clear-cut evidence that the nitrosation of phenolic compounds is much easier than that of most secondary amines. The latter are present in the digestive tract largely as their conjugate acid species (R_2NH_3^+) and are therefore unreactive, whereas phenol, for example, undergoes nitrosation readily by a pH-independent pathway from pH 1 to 5¹⁰. This difference is illustrated by comparable pseudo first-order rate coefficients (rate = k (substrate)) at pH = 1.5 and 25° C for the interaction of dimethylamine¹¹ (to form N-nitrosodimethylamine) and phenol (B. C. C. and R. J. Higgins, unpublished) (to form *p*-nitrosophenol) with 3×10^{-3} M sodium

nitrite (Table 1). It is evident that phenol reacts about 10^4 times more rapidly than dimethylamine. This large difference should prevail for most alkylamines and be virtually independent of acidity up to pH = 5; it should be even greater for polyhydroxylated (for example catechol) and polycyclic (for example 2-naphthol) aromatic compounds, both of which are more reactive towards nitrous acid than phenol. Further, the dietary intake of phenolic compounds is probably much larger than secondary amines, because the former are common constituents of both plant (flavones, quinones and so on) and animal (adrenaline, tyrosine, tryptophan and so on) material. Of possibly greater significance is the estimated 42 mg–300 mg of phenolic material produced each day by the stomach via the gastric juice¹².

Table 1 Nitrosation Rates for Dimethylamine and Phenol at pH=1.5 and 25° C

Substrate	$10^2 \times k^*$	$10^4 \times k^\dagger \text{ min}^{-1}$
Dimethylamine	$1.88 \text{ min}^{-1} \text{ mol}^{-1} \text{ l}^2$	0.0019
Phenol	$65.4 \text{ min}^{-1} \text{ mol}^{-1} \text{ l}$	20

* For dimethylamine, $v=k$ (total dimethylamine) $[\text{HNO}_2]^2$; for phenol $v=k$ (total phenol) $[\text{HNO}_2]$.

† From k assuming $[\text{HNO}_2]=3 \times 10^{-3}$ M, close to the expected concentration in the human stomach for foodstuffs containing the statutory US NaNO_2 limit of 200 p.p.m.

It is evident that the implications of using nitrite salts as a food preservative are wider than their relation to the formation of secondary N-nitrosamines. Conversely, if nitrosophenols prove to be harmless, then phenolic compounds present in both the diet and the gastric juice may effectively remove nitrite salts before they have a chance to interact with any secondary amines or other nitrogenous material from which N-nitrosamines may form.

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Received May 8, 1973.

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Reference Abbreviations

FROM September 1 it is *Nature's* intention that all abbreviations of references should conform to the style of the *World List of Scientific Periodicals*, fourth ed. (Butterworth, 1963–65). The changeover will be gradual and authors submitting manuscripts from now on are asked to ensure that the references are written appropriately.

BOOK REVIEWS

The Roots of Ethology

The Animal in its World: Explorations of an Ethologist, 1932-1972. By Niko Tinbergen. Volume I. *Field Studies*. Pp. 343. (George Allen and Unwin: London, February 1972.) £6.35.

THIS delightful book needs no praise, for it stands firmly upon its obvious merits and upon the reputation of its author. It consists of reprints, or translations, of ten of Niko Tinbergen's well known studies of animal behaviour in the field, spanning forty years of investigation (1932-72). Some of these papers are "classic" and will already be known to all well-read ethologists. But not all of them are now easy of access, and it will be a great convenience to many ethologists to have them readily available in this new format. There is another great advantage, however, in the production of this volume—this is that the papers are so readable and intriguing, in a way so delightfully simple and yet so profound and, above all, so redolent of the open air, that there are innumerable field naturalists and other non-specialists who will find them eye-opening as well as refreshing and stimulating to the mind.

The four earlier papers deal with the behaviour of two insects—the bee-hunting digger wasp (*Philanthus*) and the grayling butterfly (*Eumenis*). Three others are concerned with the black-headed gull, one with comparative studies of several species of gull and in addition there is a delightful study of food-hoarding by the fox. Finally there is a consideration of the advantages to small defenceless animals of living "scattered" rather than in crowded groups or colonies.

But in addition to all this the book is of interest to historians of biology in that it displays the research course followed by one of the pioneers of ethology. The dust cover links Niko Tinbergen and Konrad Lorenz as jointly the founders of "the young science of ethology". In a sense this is true in that both of them have been prime movers, in the period of forty years covered, in securing the establishment of ethology as a separate, respectable and inevitable branch of animal biology. It is also quite clear, from what both Tinbergen

and Lorenz have from time to time said, and indeed from what the former says in this book, that though indeed originators they are also in large measure canalizers. Many of the essential concepts of modern ethology can be found long before these two came upon the scene in the writings of Jakob von Uexküll and Oscar Heinroth in Germany; of Lloyd Morgan and Julian Huxley in Great Britain; and still more remarkably of C. O. Whitman, Wallace Craig, William Morton Wheeler and Karl Lashley in the USA. Yet Konrad Lorenz is rightly regarded as the founder of "modern ethology" in that he alone succeeded in welding these concepts together to form an effective general theory of animal behaviour. And it was largely due to his doing this that ethology graduated from being a conglomeration of isolated, albeit fascinating, studies of the field biology of individual animals, to become a really comparative science. So it is interesting to see in this volume the change of emphasis that occurs in Niko Tinbergen's work after the critical year 1938, when he and Lorenz first worked together. That brief period of collaboration in Altenberg, Austria, was rudely shattered by the coming of the Second World War, which in due course sent Konrad to a prison camp in Russia and Niko to a German hostage camp in Holland. And it was ten years before they came together again on a moving occasion in Cambridge.

From then on much of Tinbergen's work consisted, in fact, of field and experimental demonstrations and investigations of many of the features in the Lorenzian system which were, in Konrad's case, based on immensely perceptive observation of animals and his ability to "know" a great variety of species, rather than on experimental studies undertaken by himself.

The fascinating early studies of Tinbergen on insect behaviour stem more from that remarkable "Baltic Baron" Jakob von Uexküll, the title of whose great book, *Umwelt und Innenwelt der Tiere*, is, as near as makes no matter, the same as that of the book we are reviewing. Equally important as forerun-

ners were Karl von Frisch in Austria and Mathilde Hertz in Germany and later England. It was these three, rather than Heinroth and Julian Huxley, who were the chief "mentors" of the young Tinbergen. But for the historian of science, the great puzzle is—why did American zoologists, having been shown the way so clearly by their own great pioneers, require a transfusion of blood from Europe before they could really get under way? For the USA could easily have been first in the field. Thus in 1898 Charles Otis Whitman^{1,2} said "Instincts and organs are to be studied from the common viewpoint of phyletic descent." Not only did he say this, he also systematically studied and evaluated instincts as taxonomic characters. He did this notably with the doves (*Columbidae*) and also extensively with the leech, *Clepsine*, and the mud-puppy, *Necturus*. Then in the ten years from 1908 Wallace Craig produced seven or eight seminal papers. Yet when I was in Harvard in 1951-2, giving a course of lectures covering modern developments of animal behaviour studies, a much aged Wallace Craig was present. But as far as I could find out, only two people in a large audience knew who he was and what he had done; and only one of them knew that he had been living in Cambridge, Mass., for years! Again in 1902 that great entomologist William Morton Wheeler wrote³ "The only term hitherto suggested which will adequately express the study of animals with a view to elucidating their true character as expressed in their physical and psychical behavior towards their living and inorganic environment, is ETHOLOGY". And fifteen years later he wrote a brilliant chapter on instincts⁴. (Not published until 1931.) Yet coming even from him, the greatest living authority on the instinctive behaviour of ants and other social insects, the seed fell on stony ground! Again J. B. Watson in 1908⁵ produced a classic monograph on the behaviour of noddly and sooty terns; and later Watson and Karl S. Lashley⁶ collaborated on the same subject. Then in 1938 Lashley⁷ produced a famous paper which con-

tained almost all the central ideas of the Lorenzian ethology, but piecemeal and not properly coordinated. The more we look into this the deeper the mystery seems to be. But I think one can suggest at least one clue, which perhaps gives us the answer. From 1898 to 1911 Thorndike, primarily a psychologist, produced and elaborated his study based upon observations of cats in "puzzle-boxes", entitled "An experimental study of the associative processes in animals." This led to a theory of animal behaviour which was quickly taken up by J. B. Watson and became known as "behaviourism". This was fundamentally associationist or "mechanist" in outlook and prided itself upon being purely objective—with the implication perhaps that it was more nearly a physiological than a psychological system. (This claim to objectivity was also a characteristic of Tinbergen's writings on ethology after his first contact with Lorenz.) I think perhaps we should not have heard so much of Watson's behaviourism had it not been that, within a few years of its publication, the work of Pavlov and Bekhterev began to be known outside Russia. And so for the first time there seemed to be a physiological system that could be related in detail to the superficially physiological ideas behind behaviouristic psychology. Behaviourism was in some ways a laudable attempt to be objective and purge behavioural study of the uncritical anecdotal writing previously so much in evidence; but behaviourism as a general theory of animal behaviour was woefully inadequate—a fact of which anyone must be convinced who tries today to read Jacques Loeb on tropisms, taxes and the like. But I think the tremendous success and obviously immense importance of Pavlovian conditioning in the form in which it struck the United States through Watson, but from which his old colleague Lashley vigorously dissented, may well explain the prolonged period of hibernation of the indigenous American ethology.

W. H. THORPE

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Survey of Syntheses

Theory and Experiment in Exobiology. Edited by Alan W. Schwartz. Volume 2. Pp. 146. (Wolters-Noordhoff: Groningen, The Netherlands, 1972.)

THE levels of argument and explanation in this serial publication suggest that its object is to inform experts about novel concepts and observations relevant to the history, nature and distribution of life. The editor might with advantage follow the example set by editors of other "Annual Reviews" and assume that readers are already familiar with background information. Textbooks and semi-popular articles could cater for the needs of those who are less well informed. If the editor feels that this serial must cater for the needs of all, a great deal of space would be saved if each issue contained a catalogue, or syllabus, of subjects about which knowledge is being assumed in the main body of the text: for example, that there are probably many planets like this one; that complex molecules such as amino acids and purines are formed by non-biological processes in reducing environments; that they will polymerize spontaneously; and that it is possible to imagine a form of "evolution" by selection among molecules during the probiotic phase of a planet's history. If something like that were done, material scattered here over about 50 pages could be compressed into two or three. Such a plan would not only save space, it would also encourage contributors to think more clearly about the novelty of what they were saying. General surveys and syntheses have their place—a new generation is always coming along that has to be told the old stories—but they are out of place in a regularly-appearing review journal. Possibly the editor has an inkling that all is not well. Hitherto, articles were solicited, now "... a cautious invitation (is extended) for contributed material from readers of this series. It is suggested that potential contributors outline their proposed article by means of a letter before undertaking it".

Three articles deal specifically with exobiology. Alexander complains that the possibility of back-contamination if samples are returned from a landing on Mars "has received essentially no consideration. . . ." It may be that the proposed quarantine routines are inadequate, and that a more extensive menagerie and garden should be used to test for dangerous organisms, but scientists and science fiction writers recognized the risk long ago. Mitz describes recent developments in automated life-detection systems to be used on Mars. These will probably be adequate provided any organisms there are so considerate as to use biochemical systems similar to our own. Steinman

is a little more broadminded and, like Pfüger a century ago, directs attention to the cyanides.

The other three articles deal with the origins of life. Allen suggests that we now have a macromolecular obsession, and that life-like activities among substances with molecular weights around 500 (which he calls bions) should be considered. So far, his attempts to detect such activities have failed. Lacey and Fox argue that origins are to be sought among proteins or proteinoids, that RNA preceded DNA, and that the original "genetic code" made stereochemical sense but has become a "code" as a result of selection for precision. I liked their gnomic comment: "Concepts must, however, be logical, whereas new facts are not required to meet such a standard." Hall, Cammack and Rao think that the ferredoxins were important enzymes during the anaerobic phase of earthly life, and that evidence about the early course of evolution can be got by comparing their amino acid sequences. This concept leads them to a plausible system of phylogeny and gives some support to the idea that eukaryotes evolved by symbioses among prokaryotes.

N. W. PIRIE

More Environment

The Science of the Total Environment. Edited by E. I. Hamilton. Volume 1. No. 1. Pp. 115. (Elsevier: Amsterdam, May 1972.) Dfl 91.00, \$26.75 (published quarterly).

A NEW journal of the "environment" perhaps needs some justification in view of the quite prolific literature now existing on this nebulous but popular topic. It is a pity that *the science of the total environment* (the lower case presumably indicating the "key" in which contributions are intended to be written) does not have an introduction in the first issue expounding the intended principles and scope of the journal. As it stands, almost any article on the physical or life sciences could be accepted, yet many of the contributions indicate a considerable specialization within the topics discussed which seems at variance with this principle of generality. Nevertheless, the eminence of the editorial board means that papers of high quality are to be expected in this journal and the issues, so far, live up to this hope.

Several papers appear on trace element analyses, notably an extension of Samsahl's classical work on automatic chemical activation analysis and a valuable progress report by the editor on a detailed study of problems in trace element analysis. In a totally different area, a wide ranging review of potential chemical hazards from drugs, feed additives, pesticides and industrial

agents is presented by Fishbein and Flann. Other articles range from measured biological effects of pesticides and similar substances to various chemical and physical aspects of air and water pollution.

Altogether the new journal seems a worthwhile venture provided it does not become submerged in a flood of similar publications. L. SALMON

Exhaustive Physics

Handbuch der Physik/Encyclopedia of Physics. Herausgeber S. Flugge. Gruppe 3: Mechanisches und Thermisches Verhalten der Materie. Band 6a, Teil 2: Festkörpermechanik 2/ Mechanics of Solids 2. Edited by C. Truesdell. Pp. x+745. (Springer: Berlin and New York, 1972.) 288 DM; \$91.30.

THIS large volume contains six articles dealing with various aspects of the theory of elasticity and closely related topics. It is presumably intended to supplement Vol. 6 of the "Encyclopedia of Physics" entitled "Elasticity and Plasticity" which was published in 1958. In addition to an article by Sneddon and Berry on classical elasticity, that volume contains contributions on photoelasticity, inelastic continua, rheology, fracture and fatigue. The present volume, in contrast, is concerned exclusively with theoretical solid mechanics, and almost entirely with elastic solids.

All of the contributions are substantial, but two of them are major works dealing exhaustively with important branches of the subject, and amounting to considerable monographs in themselves. The first of these is by M. E. Gurtin and is entitled "The linear theory of elasticity". Professor Gurtin states his purpose as being to "give an exhaustive presentation of the linear theory of elasticity", and he proceeds to do exactly that. His words, however, are intended to be interpreted strictly literally; the treatise is concerned exclusively with the development of the theory and the statement and proof of theorems in it. Applications play no part whatsoever in the presentation. The main chapters of the article deal respectively with the formulation of the linear theory of elasticity, elastostatics, and elastodynamics. The treatment is certainly exhaustive; every theorem and result in linear elasticity of which I am aware is included, and very many more besides. Even more pleasing is the clarity of the presentation. Professor Gurtin has achieved the rare feat of being simultaneously rigorous and readable. This can only be described as a brilliant account of linear elasticity theory which will surely become the standard reference work in that subject for many years to come.

The other long article is by P. M. Naghdi on the theory of plates and shells. The first problem which confronts any writer on this subject is to decide whether to develop the theory by the direct approach, in which the shell is treated as a directed two-dimensional deformable surface, or by approximations from the equations for three-dimensional deformable bodies. Professor Naghdi resolves this dilemma by developing both formulations in parallel. This approach gives many insights into the theory, and invites interesting comparisons of these two ways of formulating it which the author does not neglect to discuss. Inevitably, this dual formulation adds considerably to the length of the article, so that at times the rate of progress seems a little slow. The main chapter headings are kinematics of shells and plates, basic principles for shells and plates, elastic shells, linear theory of elastic plates and shells. There is an appendix on the geometry of a surface and related results. The theory developed is a thermodynamical theory. A complete account is given of elastic plates and shells, but there is very little reference to inelastic materials. This article also is concerned exclusively with the development of the theory, and includes no discussion of applications. It collects together much of the recent work on the subject, as well as some not previously published, and will be a valuable work of reference for all who are interested in the foundations of the theory of plates and shells.

Similar comments can be made about the shorter, but still substantial, article by S. S. Antman on the theory of rods. This also formulates the theory both by approximation of three-dimensional equations and as a director theory. Some problems for non-linear elastic rods, mostly of a rather general nature, are also discussed. The article by D. E. Carlson on linear thermoelasticity follows that by Gurtin both in the book and in spirit. Although likely to be overshadowed by its predecessor, it nevertheless gives a clear and rigorous account of its subject and is a welcome contribution to the volume. The remaining two articles (the shortest two in the book) are both by G. Fichera and deal with existence theorems in elasticity. They are very mathematical in character even by the standards of this volume. Although they are presented in the context of linear elasticity theory, they deal with much more general problems in the theory of partial differential equations, and require a good knowledge of functional analysis from the reader.

The book as a whole will no doubt become a valued reference work, as an encyclopaedia should. The individual articles are excellent in their various

ways, but they do not together amount to anything approaching a comprehensive account of elasticity. The emphasis throughout is very strongly on formulation and general theory. Applications are hardly anywhere mentioned, and a newcomer to the subject looking through the volume would scarcely be likely to realize that elasticity is, for good reasons, of interest to engineers and applied physicists. He might even wonder why it forms part of an encyclopaedia of physics. However, this is not a book for beginners, and it must be presumed that potential readers will have sufficient background knowledge to be able to recognize that there are many other aspects to the subject of elasticity besides those presented here, and will place the individual articles of the volume within this wider context. Such readers will find it a most useful addition to the literature, although few of them are likely to be able to afford to purchase it for themselves.

A. J. M. SPENCER

Gunn Diodes

Transferred Electron Devices. By P. J. Bulman, G. S. Hobson and B. C. Taylor. Pp. x+402. (Academic: London and New York, November 1972.) \$26.50.

THIS book covers a substantial amount of background information on the microwave semiconductor devices commonly known as Gunn diodes. These devices depend on the transfer of electrons from a low mass, high mobility condition to a high mass, low mobility condition, when elevated electric fields are applied to n-type gallium arsenide, to generate or amplify microwaves.

The devices are already being widely used in broadly swept microwave oscillators, as low noise local oscillators, as power sources for intrusion alarms; and pulsed versions have been developed for limited range radars. The increase in the use of these devices from year to year shows that extremely high production volume of them will eventually exist.

The information presented in the book covers the physical fundamentals, the crystal growth and processing technology, the device-circuit interaction, and general and special applications of the devices. The level of the presentation is such that upper university level students and working professionals can easily learn the subject for the first time. It is also interesting reading and a valuable reference for anyone who is an expert in some aspect of the field. Such an expert will notice that recent results could not be included because of the usual publication delays. This is an inevitable problem in a fast moving field. It is now ten years since the first experimental results were obtained on

these devices and thus it is necessary to document the ideas and the progress to help the many people now involved in the field.

The authors are to be especially praised on two aspects of the book. One aspect is the inclusion of numerous references, with an author index that includes nearly 300 names. The continuing development of this field will be carried out by many of the authors listed, so that a newcomer to the field will know who to contact for new information. The other aspect is that the authors made a strong, extra effort to become knowledgeable about those specialties of the field in which they had little experience. As a result, the total presentation is uniform and solid in all areas.

I highly recommend the book, especially to all those people new to the field, and would suggest that it be used in advanced courses on the subject.

L. F. EASTMAN

Ring Systems

Inorganic Rings and Cages. By D. A. Armitage. Pp. 387. (Edward Arnold: London, December 1972.) £12.50.

THIS book will inevitably invite comparison with Allcock's *Heteroatom Ring Systems and Polymers* (Academic Press, New York and London, 1967), and the exhaustive two-volume treatise by Haiduc, *The Chemistry of Inorganic Ring Systems* (Wiley, New York and London, 1970). Dr Armitage has confined himself to systems, rings or cages, which do not contain carbon, in contrast with the book by Allcock which, on the other hand, excludes homoatomic systems. The volume under review is more selective but less comprehensive than the one by Haiduc. It will therefore be up to the individual reader to decide which of these three volumes he prefers, as undoubtedly economic necessity will dictate some selectivity. This volume is of course the most up to date of the three and goes systematically right through the main group elements. I found the book most interesting and well worth reading. The literature is covered up to 1970 and each chapter contains an appendix including references from the 1971 literature. Obviously in a book of this size, there must be omissions and I noticed some, particularly in the fields with which I am most familiar. The volume is not always fully referenced and some of the references are placed in such a manner as to be misleading. Quite a number of the names in the reference lists have been misspelt. The book contains a formula index, a specific index of compounds, as well as a general index, but no author index. It is a stimulating, worthwhile book

in a field which seems to have begun again to attract the amount of attention it really deserves. This and the other two comparable books all have their merits and are worth buying. Individual research workers and teachers will have to decide for themselves which of these most meets their specific needs.

R. A. SHAW

Crystal Defects

Defects in Crystalline Solids. By B. Henderson. Pp. xi+203. (Edward Arnold: London, December 1972.) £6 cloth; £3 paper.

THIS is the first book to appear in a series entitled *The Structures and Properties of Solids*, edited by Professor B. R. Coles. The general level is appropriate for mature undergraduates and junior postgraduates in physics, chemistry, metallurgy and materials science. Dr Henderson's text is based on lectures delivered at the University of Keele, and is based towards point defects and small clusters rather than, as tends to be more common in books on defects in solids, towards dislocations and mechanical properties. Following the first chapter, which gives a general introduction to defects, five of the remaining seven chapters discuss point defects in ionic solids, semiconductors and metals. The final chapter describes the interaction of dislocations with other defects, and covers some aspects of slip and hardening. Defects induced both thermally and by radiation are discussed, although there is very little on radiation damage mechanisms as such.

The general layout of the book is good and the material chosen covers a wide range of defect properties. The principal criticism is that there is a large number of misprints and mistakes in the text and equations, which detracts from the usefulness of the book to a beginner in the subject. The experienced reader will easily notice the occasional error, but someone less familiar with the subject could have a frustrating time in places trying to sort his way through mixed notations and inconsistent equations. In one or two places the text is also a bit misleading. For example, the description of the formation of F_A centres in alkali halides is at variance with the presently accepted mechanism. How many of these shortcomings are due to the author and how many to the publisher is hard to decide, but in any event it is a great pity that an otherwise excellent book should suffer in this way.

The book should prove attractive to readers interested in receiving an introduction to the science of defects in solids, and it is to be hoped that a second edition will be produced with the errors corrected. A. E. HUGHES

Sulphydryl Biochemistry

Biochemistry of the SH Group: The Occurrence, Chemical Properties, Metabolism and Biological Function of Thiols and Disulphides. By P. C. Jocelyn. Pp. xx+404. (Academic: New York and London, November 1972.) £6.50; \$20.

THIS small book sets out to cover a very large field indeed. It deals with the properties and functions of low molecular weight thiols and disulphides; with SH and SS groups in proteins; with the occurrence and functions of these groups in various mammalian tissues, in microorganisms and in plants; and concludes with chapters on radioprotection and pharmacology. The author states that the book is intended primarily for first year university students. However, it is difficult to see how he could get so much information into one small volume in a form which is intelligible to students.

The first 100 pages cover the chemical aspects of the subject. After describing a wide range of naturally occurring thiols and disulphides (including proteins) the author goes on to deal with their physical properties, chemical reactions and methods of estimation. These chapters are good in parts but unfortunately one has to know the subject to know which parts are good. Thus Hopkin's early work on glutathione is given in some detail but the section on analytical methods is unbalanced and fails to give the reader a proper understanding of the principles on which the different methods are based. Equally, hydrogen bonding of sulphur and thiazoline ring formation, which cannot explain the phenomenon of the unreactive SH group, are given the same weight as hydrophobic bonding, which can.

The latter half of the book, which covers the biological aspects of the subject, suffers from the same deficiencies. Thus the chapter on thiols and disulphides in blood is adequate but the following chapters on their occurrence in animal and plant tissues are little more than lists of headings. The last two chapters on radioprotection and pharmacology could well have been omitted from a book of this level.

The bibliography, containing some 2,000 references, is large for a book of this size but inadequate for the field that is being covered. A sample check showed that one in five of the references contains errors (I am given two sets of initials).

This book could have been very valuable for students if it had covered a more limited field in greater detail and, above all, more critically. In its present form it is misleading for students and inadequate as a source of reference for research workers. R. CECIL

CORRESPONDENCE

Nerve Growth Factor

SIR,—Drs Hendry and Iverson (*Nature*, **243**, 500; 1973) report that surgical excision of submaxillary salivary glands in adult mice causes a profound temporary drop in the plasma and tissue concentration of nerve growth factor, which was followed by a fairly rapid recovery to approximately normal plasma concentration. They postulated that an unidentified source of NGF must have been available which was capable of maintaining normal plasma concentrations. Furthermore, it is known that NGF is absent from the mouse sub-

maxillary glands during foetal and pre-adolescent life and attempts to isolate it from the glands of animals other than the mouse have been unsuccessful, and yet it is essential for the growth in culture of both sympathetic and sensory ganglia of many species. It would, therefore, be expected that another source of NGF should be available to foetal and pre-adolescent mice and other species of animal. Last year (*Exp. Cell Res.*, **74**, 170; 1972) we reported that fluorescent labelled anti-NGF stained intestinal mucosae of all the foetal and adult animals belonging to many species, including mice. In gel-diffusion the

crude mouse intestinal homogenate produced two lines of precipitation against anti-NGF, one of which showed a reaction of identity with NGF. Although all our attempts to demonstrate nerve growth promoting activity in the crude homogenates were unsuccessful, it is possible that a sensitive assay technique like radioimmunoassay may be able to detect its presence in the intestine.

Yours faithfully,
S. KUMAR

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Obituary

Mikhail Dmitrievich Millionshchikov

ACADEMICIAN MIKHAIL DMITRIEVICH MILLIONSHCHIKOV, vice-president of the Soviet Academy of Sciences, deputy director of the Kurchatov Institute of Atomic Energy, and a leading spokesman for the Soviet Union regarding scientific contacts with the West, died on May 27, 1973.

Millionshchikov was born on January 15, 1913, in Grozny in the Caucasus. He was educated at the Grozny Petroleum Institute, where he graduated in 1932. By the time he was twenty, he had already attracted considerable notice with his research on problems of the filtration of oil and gas through rocks. In 1934, he became an instructor at the Moscow Aviation Institute, where he remained until 1943. Here he carried out research into the theory of turbulence, working initially under the supervision of the mathematician Andrei N. Kolmogorov, and obtaining some now classic results.

In 1943 he became an instructor at the Moscow Institute of Engineering and Physics and worked simultaneously at the Institute of Mechanics of the Soviet Academy of Sciences. He joined the Soviet atomic energy programme in 1949, working at the Kurchatov Atomic Energy Institute on various aspects of nuclear engineering, notably the direct conversion of nuclear energy into elec-

trical energy. In 1960, on Kurchatov's death, he became deputy director of the Institute, continuing his research on nuclear reactors, direct conversion technology and low temperature plasmas.

He was elected a full member of the Academy of Sciences in 1962, becoming a vice-president of the academy in the same year. From that time onwards, he was greatly involved with organizational and training problems, and was especially concerned with the training of young scientists, the establishment of new science centres of the academy and the decentralization of research throughout the Soviet Union. In 1966, he became editor of the *Vestnik* of the academy.

Millionshchikov was a keen supporter and participant in the Pugwash movement, attending the first meeting in 1957 and becoming Chairman of the Soviet Pugwash Committee in 1964. In 1967 he became chairman of the Supreme Soviet of the Russian Federated Republic, and in both capacities was in an unusually privileged position for maintaining and strengthening scientific contacts with other countries. As early as 1970, he spoke publicly in favour of Soviet American cooperation in space.

Millionshchikov's published works are relatively few, but his contribution to research in recent years, although classified, won him Lenin and State Prizes, and many Soviet honours and

awards, including the Order of Lenin (four times) and the title of Hero of Socialist Labour (the highest Soviet civil award) in 1967. He also won several awards from other countries and was an honorary member of the Academy of Sciences of the German Democratic Republic and of the American Academy of Arts and Sciences.

Announcements

International Meetings

September 10–12, **First European Fluid Power Conference**. (The Secretary, First European Fluid Conference, National Engineering Lab., East Kilbride, Glasgow G75 0QU, Scotland.)

September 10–12, **Third Biennial Symposium on Turbulence in Liquids**. (Walter Ries, Continuing Education, University of Missouri-Rolla, Rolla, Missouri 65401, USA.)

September 10–12, **Conference on Differential Geochemistry and Its Applications in Engineering**. (The Secretary and Registrar, Institute of Mathematics and Its Applications, Maitland House, Warrior Square, Southend-on-Sea, Essex SS1 2JY.)

September 10–13, **First National Quantum Electronics Conference**. (Dr R. C. Smith, Honorary Secretary, IOP Quantum Elec-

tronics Group, Electronics Department, The University, Southampton SO9 5NH.)

September 10–14, **Sixth International Mass Spectrometry Conference.** (C. H. Maynard, Administrative Secretary, Institute of Petroleum, 61 New Cavendish Street, London W1M 8AR.)

September 10–14, **Radioimmunoassay and Related Procedures in Clinical Medicine and Research.** (International Atomic Energy Agency, Karnner Ring 11–13, A-1011 Vienna, Austria.)

September 10–14, **IUPAC International Symposium on Macromolecules.** (Dr John F. Gibson, The Chemical Society, Burlington House, London W1V 0BN.)

September 10–16, **Seventh Congress of the International Union for Study of Social Insects.** (Dr P. E. Howse, Department of Zoology, The University, Southampton SO9 5NH.)

September 10–20, **Third International Congress for Virology.** (Professor P. Wildy, Department of Virology, Medical School, University of Birmingham, Birmingham.)

September 10–21, **Ambident Reactivity of Conjugated Systems.** (Dr P. W. Hickmott, Department of Chemistry, University of Salford, Salford M5 4WT.)

September 11–14, **Non-Silver Photographic Progress.** (Mr C. J. V. Roberts, Research Labs., Kodak Ltd, Welldstone, Harrow, Middlesex.)

September 11–14, **British and German Pharmacological Societies Joint Meeting.** (Professor Dr Hans Herken, Pharmakologisches Institut der Freien Universität Berlin, 1 Berlin 33, Thielallee 69/73, Germany.)

September 12–15, **Sixth Congress of the Organ Transplantation Society.** (Organizing Secretariat, Studio MGR, Via Lanzine 40, I-202123, Milan, Italy.)

September 13–15, **International Orthopaedic Congress in Rotterdam.** (The Secretariat, c/o Holland Organizing Centre, 16 Lange Voorhout, The Hague, The Netherlands.)

September 13–15, **International Congress on the Knee Joint.** (Secretariat, c/o Knee Joint Congress, Medical Faculty of the Erasmus University, 50 Dr Molenwaterplein, Rotterdam, The Netherlands.)

September 16–20, **International Conference on Silicon Carbide.** (Professor J. W. Faust, Jr, College of Engineering, University of South Carolina, South Carolina 29208, USA.)

September 16–21, **Fifth International Fundamental Research Symposium.** (The British Paper and Board Makers' Association, Technical Section, Plough Place, Fetter Lane, London EC4.)

September 16–21, **Sixth Harden Conference.** (The Executive Secretary, The Biochemical Society, 7 Warwick Court, London WC1R 5DP.)

September 17–19, **BAGG Annual Conference.** (Dr J. C. Brice, Mullard Research Labs., Redhill, Surrey, England.)

September 17–20, **UK Automation Council, 5th Control Convention.** (The Conference Secretary, The Institute of Measurements and Control, 20 Peel Street, London W8.)

September 17–21, **International Chemical Conference.** (Dr E. R. Braithwaite, Climax Molybdenum Co. Ltd, Villiers House, 41–47 Strand, London WC2N 5JS.)

September 17–21, **Combustion Institute European Symposium.** (Dr A. R. Jones, Department of Chemical Engineering and Chemical Technology, Imperial College, London SW7.)

September 17–21, **Instrumentation, Control and Automation of Wastewater Treatment Systems.** (Mr A. Chandler, Secretary, UK Organizing Committee, Automation Workshop, c/o Greater London Council, Department of Public Health Engineering, 10 Great George Street, London SW1P 3AB.)

September 17–22, **International Biological Programme.** (Dr A. J. Holding, Department of Microbiology, School of Agriculture, University of Edinburgh, West Mains Road, Edinburgh EH9 3JG.)

September 18, **Autumn Conference and Annual General Meeting.** (The Association for High Speed Photography and the British National Committee for High Speed Photography, c/o Central Unit for Scientific Photography, Royal Aircraft Establishment, Farnborough, Hants.)

September 18–20, **Intermediates in Electrochemical Reactions.** (The Assistant Secretary, The Faraday Division, The Chemical Society, Burlington House, London W1V 0BN.)

September 18–20, **Electrofocusing Symposium.** (Dr J. P. Arbutnot, Dept. of Microbiology, The University, Alexander Stone Building, Garscube Estate, Bearsden, Glasgow.)

September 18–20, **Conference on Mathematics in Clinical Medicine.** (Secretary and Registrar, The Institute of Mathematics and Its Applications, Maitland House, Warrior Square, Southend-on-Sea, Essex SS1 2JY.)

September 18–21, **Sixth International Meeting on Organic Geochemistry.** (Keith A. Kvenvolden, Chief, Chemical Evolution Branch, National Aeronautics and Space Administration, Ames Research Center, Moffett Field, California 94035, USA.)

September 18–21, **Conference on High Voltage Direct Current Transmission.** (The Institution of Electrical Engineers, Savoy Place, London WC2R 0BL.)

September 18–21, **Pore Structure and Properties of Materials.** (Technical University of Prague, Building Research

Institute, Symposium RILEM/IUPAC Office, Solinova 7, 16000 Prague 6, Device, Czechoslovakia.)

September 19–20, **Conference on Environmental Engineering Problems in Industrialized and Developing Countries.** (Mrs Ann Cook, Conference Manager, Savoy Place, London WC2R 0BL.)

September 19–21, **Polymer Physics Group Biennial Meeting.** (Dr B. Jennings, Dept. of Physics, Brunel University, Uxbridge, Middlesex.)

September 19–21, **Colloid and Surface Chemical Aspects of Lubrication and Lubricants.** (Professor R. H. Ottewill, School of Chemistry, University of Bristol, Bristol BS8 1TS.)

September 19–21, **Biennial Polymer Physics Conference.** (Polymer Physics Group of the Institute of Physics, 47 Belgrave Square, London SW1X 8QX.)

September 19–23, **Oak Symposium.** (Mrs Mary Briggs, M.P.S., White Cottage, Slinford, Horsham, Sussex RH13 7RG.)

September 20, **Communities through Time.** (Dr J. E. Pollard, Geology Department, The University, Manchester M13 9PL.)

September 20–21, **Organization and Function of Microtubules and Microfilaments.** (Secretary of the Second International Congress on Cytopharmacology, Department of Pharmacology, University of Milan, Via Vanvitelli 32, 20129 Milan, Italy.)

September 20–21, **Symposium on Insect Flight.** (The Society's Registrar, 41 Queen's Gate, London SW7 5HU.)

September 20–21, **Properties of Building Materials.** (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX.)

September 20–21, **British Society for Cell Biology.** (Dr B. M. Richards, Searle Research Labs., Lane End Road, High Wycombe, Bucks. HP12 4HL.)

September 20–23, **Joint Geological Societies Meeting.** (Secretary of the Department of Geology, University of Manchester, Oxford Road, Manchester M13 9PL.)

September 20–28, **Fourth Symposium on Microdosimetry.** (Secretariat of the Fourth Symposium on Microdosimetry, Commission of the European Communities, DG XII—Biology Division, Dr H. G. Ebert, 200 Rue de la Loi, B-1040 Brussels, Belgium.)

September 21, **Meeting of the Chemical Society.** (Carbohydrate Group, Dr N. R. Williams, Chemistry Department, Birkbeck College, Malet Street, London WC1E 7HX.)

September 21–23, **Annual Conference of the British Society for the Philosophy of Science.** (Mr Larry Briskman, Department of Philosophy, David Hume Tower, George Square, Edinburgh EH8 9JX.)

September 22–27, **Fourth International Congress of Food Science and Technology.** (The Secretariat, IV Congress, Jaime Roig 11, Valencia 10, Spain.)

September 23–26, **Optical Properties of Thin Films.** (Dr H. G. Jerrard, Department of Physics, University of Southampton SO9 5NH, or Dr P. H. Lissberger, Department of Pure and Applied Physics, University of Salford M5 4WT.)

September 23–26, **ASLIB 47th Annual Conference.** (The Conference Organizer, ASLIB, 3 Belgrave Square, London SW1X 8PL.)

September 23–27, **Eighth International Conference on the Properties of Steam.** (The Secretary, UK National Committee on the Properties of Steam, 1 Birdcage Walk, London SW1H 9JJ.)

September 23–29, **International Conference on Kimberlites.** (Dr J. J. Gurney, Department of Geochemistry, University of Cape Town, Rondebosch, C.P., S. Africa.)

September 24–25, **Environmental Health Engineering in Hot Climates and Developing Countries.** (John Pickford, Department of Civil Engineering, University of Technology, Loughborough, Leics. LE11 3TU.)

September 24–27, **The Chemical Society Autumn Meeting.** (Dr John Gibson, The Chemical Society, Burlington House, London W1V 0BN.)

September 24–28, **Transduction Mechanisms in Chemoreception.** (T. M. Poynder, Secretary, Department of Physiology, University College, London.)

September 24–28, **Noble Gases Symposium** (Dr A. Alan Moghissi, Chairman of the Program Committee, Noble Gases Symposium, US Environmental Protection Agency, PO Box 15027, Las Vegas, NY 8114, USA.)

September 25–27, **First European Tri-biology Congress** (Eurotrip 73, Groups Department, The Institution of Mechanical Engineers, 1 Birdcage Walk, London SW1H 9JJ.)

September 25–27, **Computer Uses in Water Systems.** (The Water Research Association, Medmenham, Marlow, Buckinghamshire SL7 2HD.)

September 26–28, **Conference on Water Pollution Control Technology in Low Density Areas.** (Dr William J. Jewell, Department of Civil Engineering, University of Vermont, Burlington, Vermont 05401, USA.)

September 27, **Colloquium on Ion Exchange Chromatography on Amino Acids and Sugars.** (The Secretary of the Group, Dr E. Reid, Wolfson Bioanalytical Centre, Leapale Lane, Guildford, Surrey.)

Errata

IN the article "Outbreeding and Inbreeding in a Zinc-Lead Mine Population of *Armeria maritima*" by C. Lefebvre (*Nature*, 243, 96; 1973), paragraph 8, line 3 and following, should read "If total inbreeding occurs the frequency of AA in the A-type progeny will be 0.25 (*not* 0.50). The potential amount of inbreeding in the population (7%) leads to the estimate that approximately 2% (*not* 4%) of AA plants must arise in the population."

IN the article "Role of Erythropoietin from Liver and Spleen in Erythropoietin Production in the Anephric Rat" by C. Peschle, A. D'Avanzo, I. A. Rappaport, S. Russolillo, G. Marone and M. Condorelli (*Nature*, 243, 539; 1973), Group 6 in Table 1 should read "Sham operation + nephrectomy + room pressure" instead of "... + hypoxia".

IN the article "Apollo 17 'Orange Soil' and Meteorite Impact on Liquid Lava", by E. Roedder and P. W. Weiblen (*Nature*, 244, 210; 1973), editorial change of the title and deletion of the opening paragraph have resulted in some confusion as to what the theory involves. The suggested mode of origin for this unusual soil, which differed from those suggested by the other groups working on it, is by meteorite impact on liquid lava containing olivine phenocrysts. Also, section (2) should read, "The olivine phenocrysts. These effectively preclude the first and the third of the three mechanisms of origin suggested above."

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

Aspects of the Crusades: a Phased Historical Case Study in Problem Solving and Decision Making. By Michael Hamilton. (The Decisions of History.) Pp. 42. (London: Academic and Business Monographs, Ltd., 6 Brodrick Road, SW17, 1973.) 93

Rheumatism and Arthritis in Britain. Pp. 44. (London: Office of Health Economics, 1973.) 25p.

Bulletin of the British Museum (Natural History). Geology. Vol. 21, No. 2: Poscanine Occlusion in Cynodonts and Tritylodontids. By A. W. Crompton. Pp. 27–71 + 7 plates. £3.40. Vol. 22, No. 1: The Classification and Natural History of *Theropithecus* (*Sinopithecus*) (Andrews, 1916). Baboons of the African Plio-Pleistocene. By Clifford J. Jolly. Pp. 1–123 + 20 plates. £6.65. Vol. 22, No. 2: Llandovery Condonts from the Welsh Borderland. By R. J. Aldridge. Pp. 125–231 + 9 plates. £4.45. Vol. 22, No. 3: A Revision of the Redfieldiform and Perleiform Fishes from the Triassic of Bekker's Kraal (South Africa) and Brookvale (New South Wales). By P. Hutchinson. Pp. 233–354. £4.30. Vol. 22, No. 4: Lower Palaeozoic Green Algae from Southern Scotland, and their Evolutionary Significance. By Graham Francis Elliott. Pp. 355–376 + 10 plates. £1.85. Vol. 22, No. 5: The Buchanosteid Arthropods of Australia. By Errol I. White and Harry A. Toombs. Pp. 377–419 + 9 plates. £2.65. Vol. 22, No. 6: Planktonic Foraminifera from the Palaeocene-Eocene Succession in the Rakhli Nala, Sulaiman Range, Pakistan. By B. K. Samanta. Pp. 421–482 + 15 plates. £3.55. Vol. 22, No. 7: *Megistotherium*, Gigantic Hyacodont from Miocene of Gebel Zelten, Libya. By R. J. G. Savage. Pp. 483–511 + 4 plates. £1.70. Vol. 22, No. 8: Introduction to the Miocene Mammal Faunas of Gebel Zelten, Libya. By R. J. G. Savage and W. R. Hamilton. Pp. 513–527. 60p. Vol. 23, No. 1: Marsupialia, Insectivora, Primates, Creodonts and Carnivora from the Headon Beds (Upper Eocene) of Southern England. By P. E. Cray. Pp. 1–102 + 6 plates. £4.45. Vol. 23, No. 2: Some British Cerataean Harpaxodonts Belonging to the Families Procerithiidae, Cerithiidae

and Cerithiopsidae (Cerithiacea). By H. L. Abbass. Pp. 103–175 + 8 plates. £3.45. (London: British Museum (Natural History), 1972 and 1973.) 124

Department of the Environment and the Welsh Office. Structure Plans: The Examination in Public. Pp. 12. (London: Department of the Environment and the Welsh Office, 1973.) *Gratis*. 124

Rutherford: The Father of Nuclear Energy. By Professor T. E. Allibone, CBE, FRS. (The Rutherford Lecture delivered on 3 November 1972 at the Polytechnic of Central London.) Pp. 24. (Manchester: Manchester University Press, 1973.) 48p net. 124

Queen Mary College (University of London). Annual Report, Session 1971–72. Pp. 168. (London: Queen Mary College, 1973.) 124

European Invertebrate Survey. Provisional Atlas of the Insects of the British Isles. General Editor: John Heath. Part 2: Lepidoptera (Moths—Part One). Edited by John Heath and Michael J. Skelton. Pp. 3 + 102. Abbots Ripton, Huntingdon: Biological Records Centre, Monks Wood Experimental Station, 1973. Sole Agents: E. W. Classey, Ltd., 353 Hanworth Road, Hampton, Middx.) £1. 124

Third Annual Report of the Advisory Committee on Oil Pollution of the Sea Research Unit on the Rehabilitation of Oiled Seabirds. Pp. 24. (Newcastle upon Tyne: Department of Zoology, University of Newcastle upon Tyne, 1973.) 50p. 134

Department of Education and Science. Statistics of Education 1971. Vol. 2: School Leavers, C.S.E. and G.C.E. Pp. xxii + 79. (London: HMSO, 1973.) £1.50 net. 134

University of Oxford. Annual Reports, 1971–1972. Pp. 18. (Supplement No. 4 to the *University Gazette*, March 1973.) (Oxford: The University, 1973.) 50p. 134

The Gardeners' Sunday Organization. Gardens to Visit. Pp. 64. (Dorking, Surrey: Mrs. K. Collett, White Witches, Claygate Road, 1973.) 10p. 134

Extension of the Chester Beatty Research Institute Serial Abridged Life Tables to Cover the years 1966 to 1970. By R. A. M. Case and Christine Coghill. (2nd Supplement to the Chester Beatty Research Institute Serial Abridged Life Tables, England and Wales, 1841–1960.) Pp. 13. (London: The Chester Beatty Research Institute, 1973.) 134

Department of Trade and Industry. Action Against Aircraft Noise—Progress Report 1973. Pp. 22. (London: Department of Trade and Industry, 1973.) 134

Family Planning: a Guide to Methods for Field-workers, Health, Social and Welfare Workers. Pp. 28. (London: International Planned Parenthood Federation, 18/20 Lower Regent Street, 1973.) 154

Water Resources Board. The Trent Research Programme. Vol. 1: Report by the Water Resources Board. Pp. 33. (London: HMSO, 1973.) 60p net. 164

Proceedings of the Royal Irish Academy. Vol. 73, Section A. No. 5: Some Computerised Trajectories and Focal Properties of Asymmetrical Electron Lenses. By L. Jacob and M. B. O'Sullivan. Pp. 47–62. 30p. No. 6: The Analysis of Non-Exponential Decay Curves in Pulsed Magnetic Resonance. By G. E. Wardell and V. J. McBrierty. Pp. 63–68. 10p. No. 7: The Production of Condensation Nuclei and Small Ions by the Deformation of Rubber Surfaces. By T. P. Burke and M. J. Byrne. Pp. 69–89. 38p. Vol. 73, Section B. No. 4: The Ordovician Volcanic Rocks of Arklow Head, Co. Wicklow. By C. J. Stillman and D. K. Maytham. Pp. 61–78 + plates 7 and 8. 36p. No. 5: Stages in the Dissolution of the Last Ice-Sheet in Ireland and the Irish Sea Region. By J. K. Charlesworth. Pp. 79–86. 9p. No. 6: Effect of Freezing, Thawing and Fixatives on the Diameter of Fibres from Pre- and Post-Rigor Skeletal Muscles. By P. V. J. Hegarty and R. T. Naudé. Pp. 87–94. 12p. (Dublin: Royal Irish Academy, 1973.) 194

University of Warwick: Careers Advisory Board. Annual Report to Council for the year ended 30th September, 1972. Pp. 31. (Coventry: University of Warwick, 1973.) 194

The Ditchley Foundation. The Director's Annual Review for 1972. Pp. 11. (Enstone, Oxford: The Ditchley Foundation, 1973.) 194

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 265, No. 868: The Pleistocene Geology of the Area North and West of Wolverhampton, Staffordshire, England. By A. V. Morgan. Pp. 233–297 + plates 32 to 34. (London: The Royal Society, 1973.) £2.65; \$7.40. 194

National Governments and the European Communities. By Helen Wallace. (European Series, No. 21.) Pp. 104. (London: Chatham House, and PEP, 1973.) £1.25. 194

The Annual Report of the Science Policy Research Unit at the University of Sussex for the year 1972. Pp. 66. (Falmer, Brighton: SPRU, University of Sussex, 1973.) 194

Potato Marketing Board. Sutton Bridge Experimental Station, Report No. 6: Storage Design and Equipment for Environmental Control in Potato Stores. Part 2: Control of Environment. By C. P. Hampson. Pp. iii + 59. (London: Potato Marketing Board, 50 Hans Crescent, SW1, 1973.) *Gratis*. 194

Titles of Dissertations approved for the Ph.D., M.Sc., and M.Litt. Degrees in the University of Cambridge during the Academic Year 1971–1972. Pp. 45. (Cambridge: The University, 1973.) 194

Department of the Environment, London. Scottish Development Department, Edinburgh. Housing Development Notes. 2: Landscape of New Housing. (1) Background. (2) Trees in Housing. Pp. 26. (London: Department of the Environment; Edinburgh: Scottish Development Department, 1973.) 194

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- Fodder Crops. Pp. 12, 10p. Farmers Leaflet No. 7: Recommended Varieties of Maize. Pp. 8, 7p. List of Oil-Seed Rape Varieties of Maize. Pp. 8, 7p. List of Oil-Seed Rape Varieties 1973. Pp. 3. *Gratis*. (Cambridge: National Institute of Agricultural Botany, 1973.) [104]
- Widening the Choice: The Next Steps in Housing. (Cmd. 5280.) Pp. 14. (London: HMSO, 1973.) 13p net. [104]
- Projects: Architectural Association 1946-71. Edited by James Gowan. (AA Careers Series, No. 1.) Pp. 127. (London: Architectural Association School of Architecture, 34/36 Bedford Square, 1973.) £1.50 paper-back; £2 hard-back. [104]
- British Pregnancy Advisory Service. Client Statistics for 1971, with Commentary. Pp. 36. (Birmingham: British Pregnancy Advisory Service, 1973.) [114]
- The Macaulay Institute for Soil Research. Annual Report 1971-1972. No. 42. Pp. 80. (Craigiebuckler, Aberdeen: The Macaulay Institute for Soil Research, 1973.) [124]
- Countryside Commission. National Park Guide No. 10: Pembrokeshire Coast. Edited by Dillwyn Miles. Pp. x+150+38 plates. (London: HMSO, 1973.) 75p net. [124]
- Fossil Amphibians and Reptiles. By W. E. Swinton. First edition. (Publication No. 543.) Pp. ix+133 (17 plates). (London: British Museum (Natural History), 1973.) 75p. [124]
- Broadcasting to the World: Forty Years of BBC External Services. By Gerard Mansell. Pp. 8. (London: BBC, 1973.) [124]
- Tobacco Research Council. Research Paper No. 12: A Comparison of Two Smoking Typologies. By A. C. McKennell. Pp. 95. (London: Tobacco Research Council, Glen House, Stag Place, 1973.) [124]
- The Function of the University—Teaching and Research: Report of a Conference. (Universities Conference, November 1972.) Pp. 53. (London: The Committee of Vice-Chancellors and Principals of the Universities of the United Kingdom, 1973.) [134]
- Building Research Establishment Digest No. 152: Repair and Renovation of Flood-Damaged Buildings. Pp. 8. (London: HMSO, 1973.) 5p. [134]
- Ministry of Agriculture, Fisheries and Food. Technical Bulletin No. 26: Flowering Periods of Tree and Bush Fruits. (Records from the National Fruit Trials, Brogdale.) Pp. v+76. (London: HMSO, 1973.) £1 net. [164]
- Potato Marketing Board. A Commercial Assessment of Recently Introduced Potato Varieties 1972. Collated by Gwyneth M. Roodcock. Pp. 20. (London: Potato Marketing Board, 50 Hans Crescent, SW1, 1973.) *Gratis*. [164]
- The University of Aston in Birmingham. Sixth Annual Report and Accounts, 1971/1972, presented by the Vice-Chancellor. Pp. 79. (Birmingham: The University of Aston in Birmingham, 1973.) [164]
- The Commonwealth Forestry Institute, University of Oxford. Forty-eighth Annual Report, 1971/1972. Pp. 29. (Oxford: Commonwealth Forestry Institute, 1973.) [174]
- Furniture Industry Research Association. Annual Report for 1972. Pp. 24. (Stevenage, Herts: The Furniture Industry Research Association, 1973.) [174]
- Population Problems and Policies in Economically Advanced Countries. (Report of a Conference at Ditchley Park, England, September 29-October 2, 1972.) Pp. 36. (Enstone, Oxford: The Population Crisis Committee in association with the Ditchley Foundation, 1973.) [184]
- The Zoological Society of London. Annual Report for 1972. Pp. 64+6 plates. (London: The Zoological Society of London, 1973.) [184]
- The Annual Report of the Committee of Visitors of the Royal Institution of Great Britain. The Statement of Accounts and Other Reports for the year ending 31st December 1972. Pp. 67. (London: The Royal Institution of Great Britain, 1973.) [194]
- Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 265, No. 869: Pleistocene *Helophorus* (Coleoptera, Hydrophilidae) from Borislav and Starunia in the Western Ukraine, with a Reinterpretation of M. Lomnicki's Species, Description of a New Siberian Species, and Comparison with British Weichselian Faunas. By R. B. Angus. Pp. 299-326+plates 35 and 36. (London: The Royal Society, 1973.) £1.22; \$3.40. [194]
- Cotton Research Corporation. Cotton Research Reports. Uganda, 1971-72. Pp. 85. 30p. Swaziland, 1970-71. Pp. 17. 30p. (London: Cotton Research Corporation, 1972 and 1973.) [244]
- University of Oxford. Ashmolean Museum—Report of the Visitors. (Supplement No. 5 to the *University Gazette*, March 1973.) Pp. 60. (Oxford: The University, 1973.) £1. [254]
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- The Exmoor Handbook and Gazetteer. (Micro-study H1.) By N. V. Allen. Pp. 76+11 photographs. (Dulverton, Somerset: The Exmoor Press, 1973. First printed 1972.) 50p. [304]
- Fisons. Report and Accounts 1972. Pp. 32. (London: Fisons, 9 Grosvenor Street, 1973.) [304]
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- Research Supported by the Social Science Research Council, 1971/1972. Pp. 293. (London: Social Science Research Council, 1972.) 60p net. [25]
- Department of Education and Science. Library Information Series, No. 2: The Public Library Service—Reorganization and After. Pp. 29. (London: HMSO, 1973.) 47p net. [25]
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